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UNDERGRADUATE TEACHING OF CLINICAL PATHOLOGY IN THE MEDICAL SCHOOLS*

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THE object of this paper is to bring to your attention a subject in which the method of teaching has varied to a great degree in the undergraduate medical schools of this country. Its purpose will be amply fulfilled if it helps in any way to unify the teaching of clinical pathology or even points clearly to the need of this.

The importance of this to all of us, whether teachers or not, bears directly upon the development of clinical pathology as a division of medical practice and upon the recognition that will be accorded it by graduates of our medical schools. If the undergraduate medical student be thoroughly trained in the importance of the proper choice of methods and of accuracy of technic, and observes the relationship of the teaching clinical pathologist to the clinicians, he will when graduated recognize the practicing clinical pathologist as a consultant who is prepared to aid him in the study of his cases.

In the development of the modern school there was for a long time a sharp distinction between preclinical and clinical teaching. The preclinical courses were taught as pure sciences and given with little relationship to the patient. The clinical teachers were men of the older school, with little knowledge of the preclinical medical sciences as then taught. There was a gap between these two requiring to be bridged.

Within the past fifteen to twenty years, courses in clinical laboratory

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†This was to be a joint paper presented by James C. Todd, of the University of Colorado and myself. Doctor Todd, as a result of a recent illness, was unable to give the subject the thorough study that he desired and felt required to withdraw from the authorship, and from share in the responsibility for the statements set forth. I take pleasure in acknowledging, however, that as a result of valuable notes sent to me by him, some of the phraseology and thought is his and is included with his approval.

diagnosis gradually evolved; starting usually as a course in urinalysis and adding to itself other chemical and microscopic examinations. This course now embraces a systematic study of the laboratory methods used in the examination of the urine, blood, sputum, gastrointestinal contents, exudates and transudates. The subjects are considered with special reference to their bearing upon the modern methods of microscopic, bacteriologic, serologic, and chemical technic used in diagnosis.

This course offered the first link in binding the preclinical to the clinical subjects for aid in diagnosis. For many reasons it could not bring all phases of the preclinical fundamental sciences in close relationship to the patient. On account of the content of the preclinical courses they have to be taken up in a scientific way and gone into more deeply than the clinician has time or opportunity to do. The necessity of still linking more closely the first two years to the other two has continued to be felt and efforts are now being made to introduce the clinical viewpoint more thoroughly into the fundamental sciences. There must be a certain and sure foundation in the study of the normal and of the causative factors of disease for the proper building, in order that the student can be developed to appreciate the necessity of correct methods and observations in his clinical work.

In order to get as accurate an idea as possible of the present status of clinical pathology in the medical school and the trend of opinion as to its function, its content and best mode of teaching, I have studied the catalogues and have sent questionnaires to all teachers of clinical pathology in the fifty-nine four year Class A Medical Schools. We have been pleased not only at receiving as high as eighty per cent of replies, but also by the great interest manifested in the results by those concerned. There are also included in the analysis thirty-four replies to a questionnaire sent by James C. Todd to prominent clinical teachers of medicine and surgery which he has very kindly turned over to me to use.

FUNCTION OF A COURSE IN CLINICAL PATHOLOGY

Before the student enters upon the course in clinical pathology he should have completed all the standard courses in the fundamental sciences with the possible exception of pathology which should, at least in part, have been covered.

Clinical pathology should not be a course in, or a renewal of these subjects except to perfect technic. The student should have an understanding of technic, observation of the normal, with some comparison to the abnormal, before arriving at clinical pathology to perfect himself in the clinical laboratory of the abnormal. Perfection of technic in the essential tests is the first principle to be taught. If accuracy is not assured in laboratory test and observation, interpretation loses its importance. We quote Dean Emerson (Indiana), as follows: "The essential function of a course in clinical pathology is training in accuracy * * * careless technic not only injures ward work, but what is more important, lowers the efficiency of the physician in all his future practice."

More specifically it seeks to train the student thoroughly in the technic

of such laboratory methods as he will have occasion to use in his practice, and to give him a general knowledge of the technic of those which are too complex for office use. It endeavors to teach the purpose of the procedures, to make clear their limitations and to correlate the findings with the underlying pathology and the clinical evidences of disease.

This instruction should be carried out not only during the introductory course, but during the remainder of the time the student is at college, by constant supervision of his laboratory work and conclusions in regard to ward and outpatient cases to which he is assigned.

In outlining our view of the function of clinical pathology, we do not wish to imply that it is a separate and independent part of the curriculum. Indeed no subject in the medical curriculum can be separate and independent. Clinical pathology is placed by most schools under the Department of Medicine, in some under the Department of Pathology and Bacteriology, in some, divided among several departments, while in others it is recognized as a separate department. No matter where placed, it cannot at all serve its proper function without the closest and most cordial cooperation with, not medicine alone, but all the purely clinical departments, both in its teaching, and in the laboratory work that it controls in the teaching hospital.

PLACE IN CURRICULUM AND EXTENT OF COURSE

When the course in clinical pathology was introduced some years ago in the medical curriculum, it was designated as "Clinical Microscopy" and was given in the fourth year. The name and place in the curriculum has been gradually changed, so that now the course is most frequently called "Clinical Pathology," though sometimes designated "Laboratory Diagnosis," or "Clinical Diagnosis," and is introduced at the time or just before the student starts his clinical work. At present, about one-half of the whole number of schools give the course in the third year, thirty-two per cent in the second year and eighteen per cent in a course extending through part of second and third years. In 1921 only fourteen per cent gave the course in the second year. This irregularity in the introduction of the course has presented some difficulties in the acceptance of credits when a student transfers from one school to another. From observation of the catalogues, over some years, it is evident that there is a continued tendency toward starting, if not completing, the formal course in the second year.

Based on information obtained, the number of hours for the formal course, exclusive of the laboratory work done in connection with hospital assignments in the third and fourth years, varies widely in the different schools from thirty-six to two hundred and fifty-six hours. Approximately one-third of the schools give from one hundred and twenty to two hundred and fifty-six hours. One-third give ninety to one hundred and twenty, one-third under ninety hours; and of these latter there are four that give less than fifty hours. The average number of hours was about one hundred.

This extreme variation emphasizes the need of some sort of standardization, if for no other reason than to facilitate transfer of students from one

institution to another. We believe these wide discrepancies do not represent the relative amount of laboratory diagnosis taught in the respective schools, but rather the extent to which clinical pathology is recognized as demanding a separate place in the curriculum.

No definite standard can be set, but if the course is to carry out its proper function, it is preposterous to expect to cover it thoroughly in thirty-six to forty-eight hours or even sixty hours. We do not wish to criticize the schools giving these brief courses for we realize that it is quite possible that adequate instruction in the subjects covered is given in related courses. The report of the committee on Undergraduate Teaching of Medicine of the Association of American Medical Colleges in 1921 recommended that one hundred and eighty hours be devoted to this subject.

VALUE OF THE DAILY CONCENTRATED COURSE

Pedagogically speaking this question is of much practical importance to both teacher and student. Of replies received to this question 82 per cent were opposed to this form of teaching. The usual reason stated was insufficient time to study and review ground covered in the laboratory. It is difficult to state exactly what is meant by "concentration"; this may be better understood by the term "block system." One, or possibly two courses are taken up, with the exclusion of all others, until completed. We feel that for acquiring accuracy of technic a certain degree of "concentration without isolation" is essential, i.e., laboratory periods of three hours in which are included thirty to forty minutes lecture or demonstration. We understand that in colleges of liberal arts where pedagogy is usually more considered than in medical schools, the ideal arrangement is three class periods a week. Students must have time to digest what they have learned, to reflect, and to compare.

From Herman (Michigan) we have the following reply: "The danger of a concentrated course seems to me to be that the students so often "cram" the information in, get 'by' the course and promptly forget all about it, * *"

CONTENT OF THE COURSE

Replies to the question, "Do you think that the required course should be limited to such ordinary routine laboratory examinations as a general practitioner would be expected to do in his office?" were diverse and interesting.

Opinion as to what should be considered ordinary routine laboratory examinations, no doubt, varies considerably. We see no reason why the general practicing physician, when time permits, should not include in his office work such laboratory examinations as the ordinary urinalyses, including microscopic examinations for blood counts and malaria, smears for gonococci, sputum for tubercle bacilli, feces for parasitic ova, test meals, and organisms in spinal fluid. The more of this work he does on his patients the better physician he will be.

Nearly two-thirds of the clinicians thought that the technical part of the course should cover only the ordinary routine that a physician would be

able to do in his office, while about three-fourths of the clinical pathologists replied that it should cover considerably more. Both classes agreed almost unanimously that by demonstration and lectures, a knowledge of the application of the more difficult methods should be obtained by the student, from both the clinical pathologist and the clinician.

Among those in favor of "ordinary routine" Sturgis (Harvard), referring to my question, states: "The above is the exact criterion I use in selecting material to be used in my course."

Of those answering that the course should consist of more than the ordinary examinations, we wish to quote the following:

Dean Emerson (Indiana) replies, "The student will never do as much laboratory work in general practice as he is taught to do in the school. Once during his medical course, therefore, he should be required to study his cases as nearly 100 per cent well as possible."

We agree with Vogel (Columbia) that, "Ordinary routine should be especially stressed, but other more advanced methods should be brought to the attention of the student." Our opinion is that he should be taught sufficient knowledge of the more refined methods of laboratory diagnosis to enable him to assimilate their relative value. Unless considerably more than ordinary routine is included he will not be able to get a broad view of laboratory interpretation. The student should have a clear understanding of the relationship of laboratory tests to other signs and symptoms, a knowledge of the practical value of the results, and an appreciation of certain technical difficulties, not only of the ordinary simple methods, but of the more complicated procedures as well. He should also, by training, acquire sufficient knowledge to be able to read and understand current medical literature on modern laboratory diagnosis.

To ascertain more definitely which of the more complicated procedures should be made an essential part of the regular course, which merely demonstrated to the class, which made elective, and which considered sufficiently covered in other courses to be used clinically, we asked specifically about the status of ten of these procedures.

It would be difficult to draw any very definite conclusions as the requirements and conditions varied considerably in the different schools. Even where subjects are mentioned as being taken up in clinical pathology, the extent could seldom be determined. The following summary is, however, of interest:

1. General cultural methods, isolation of bacteria, typing of pneumococcus, and animal inoculation are generally considered by a large majority of the replies to be well enough covered in the course in bacteriology. Blood cultures are, however, an exception to this, for more than half the schools give this as repeated in clinical pathology; in 40 per cent, as required, and in others as demonstrated or elective.

2. Diagnostic blood chemical examinations are assigned to clinical pathology by about three-fourths of the medical schools. Nearly one-half of the schools make it a required part of the course, and in the others it is demonstrated or elective.

3. Serodiagnosis (distinguished from principles of immunology) is assigned to clinical pathology by two-thirds of the replies. Most of this, with the exception of Widal tests, is given as elective. In certain schools, however, Wassermanns are placed as required or demonstrated.

4. The colloidal gold test, determination of basal metabolism and complicated functional tests are included as either demonstrated or elective in clinical pathology by three-fourths of the schools.

5. Skin tests, as tuberculin, Schick, and foreign protein-pollen sensitization, are mentioned as being given in clinical pathology in a few schools.

Most of the above subjects are given in their fundamental aspects in other departments, the clinical importance being demonstrated in clinical pathology. The opportunity for the student to perfect himself in technic and to continue the study of them clinically should be elective in the third and fourth years under supervision of the clinical pathologist. The promotion of this encourages a keen sense for investigation and we find our best students eager to follow when encouraged and led on. It is during this period that we take the first steps in the development of our future clinical pathologist, but of course it must not be carried too far. Vogel (Columbia) writes: "* * * we are not trying to make scientist and research workers, but doctors." However, we would reply to this that we must not lose sight of the fact that if we do not instill some desire for investigation and research, medicine will not become a science, but a lost art.

The questions, "Do you think the present medical student is taught so much dependence upon laboratory methods that he neglects the working out of other clinical evidences of the cases to which he is assigned?" and "Where would you say the fault lies?" bring us to a subject that was widely discussed.

The point is, not that the student is taught too much of laboratory methods, but whether he is taught too much dependence upon laboratory methods. The opinions of the clinicians and the pathologist were quite divergent upon these questions. About two-thirds of the clinicians agreed that the student is too dependent upon the laboratory and about two-thirds of the clinical pathologists maintained that he is not. The clinician has probably the better opportunity to judge.

Dean Graves (Louisville) writes as follows: "Any person with common sense who has enjoyed a well-balanced preparation in laboratory sciences and has applied these methods to clinical medicine is in no danger of neglecting either the laboratory or the clinical signs and symptoms."

On the other hand Dean Emerson (Indiana) writes: "Undoubtedly the student leans too heavily on laboratory methods, and still more heavily on the reports of laboratory work which others do. * * *" He later adds: "This does not mean that we should teach less clinical pathology; rather a great deal more."

Bentz (Buffalo) replies: "Too much of clinical systematology is taught without proper correlation."

Some of our respondents that deny that the student is too dependent on laboratory diagnosis offer, however, an opinion as to where the fault lies. We quote the following opinion of Cummer (Western Reserve): "Too much

emphasis placed on the laboratory by teachers of the fundamental sciences in the first two years, and lack of perspective by teachers of this subject. The worst offenders, however, are the old time clinicians who either scorn all laboratory tests, or go to the other extreme of placing too much emphasis on the findings."

Keegan (Nebraska): "This depends entirely upon the organization of the clinical staff. Ignorance on their part leads either to indiscriminate use of laboratory reports, or to a belittling attitude towards all laboratory work."

Several of the clinicians agree with Christian (Harvard), who writes: "In my judgment, a common fault of medical schools is the attempt to teach the student too many of the elaborate methods of examinations whether laboratory or otherwise, with the neglect of drill in the simple methods of medicine."

Most of the replies agree that it depends upon the teachers both in the laboratories and the clinic. Students are just what we choose to make them. They can be made to take a keen interest in their work so long as the proper incentive is placed before them.

The chemist, the physiologist, the bacteriologist, and pathologist emphasize, and possibly overstress, certain points in their subjects in order that the student can more fully grasp and retain them.

The clinical pathologist, while he adds the clinical viewpoint, likewise brings forward the laboratory side of medicine. This all comes in part or entirely before the student arrives in close contact with the patients. There is much to consider in the force of first impressions.

We believe the student does depend too much at first upon his laboratory diagnosis. However, after two years in clinical work in which he is guided by the clinicians, with the cooperation of the clinical pathologist, he should have reached the point of determining the relative value of his laboratory results to the other signs and symptoms of disease. The student gets his first ideas of laboratory interpretation through the clinical pathologist and should continue to be under his supervision and receive his advice throughout his clinical years.

This is a debatable point in some schools and so the question was asked: Should interpretation of laboratory findings be taught by the clinical pathologist, by the internist, or by both?

Only two of the clinical pathologists and three of the clinicians stated that interpretation should be taught by the internist alone. The rest all agree that it should be taught by both. Sturgis (Harvard) writes: "The greatest emphasis should be placed on technic in this course. A certain amount of interpretation should be taught, but the internist has the greatest opportunity to teach this."

Kinsella (Washington) believes, "The clinical pathologist should be an internist." According to our views he should at least make rounds in the teaching hospital frequently enough to keep in contact with interesting cases and not spend all his time merely teaching in his laboratory and doing mechanical work. In some schools he acts as a clinical teacher in the hospital wards, as well as instructor in the laboratory.

A clinical pathologist is not merely a technician; there is the necessity to keep up with the clinical side of medicine, otherwise he cannot teach his subject with the due consideration of interpretation that belongs to it. This is important, since it involves the whole question of the status of the clinical pathologist. We insist that as a teacher he is even less a technician than a clinical pathologist practicing his profession outside.

Both in his teaching and his capacity as consultant, he is concerned with the indications for certain tests, with the principles of interpretation and with the exact significance of the tests in particular cases. Undoubtedly, however, the final evaluation of the laboratory findings in relation to the data obtained from all other sources must rest with the clinician.

A PROPOSED UNDERGRADUATE COURSE IN CLINICAL PATHOLOGY

It would be presumptuous in us to set down in detail the ideal course. There can in fact be no single ideal course, however well worked out, to fit the diverse conditions to be met in the medical schools. We believe there should be sufficient comparable standardization for credit in other schools, which at present is not the case. We shall, however, present an outline of a course which we believe well suited for schools like ours in which there is a teaching hospital in close physical relation with the school buildings.

It is necessary to keep rigidly in mind that we are not trying to make clinical pathologists but general practitioners. As we would plan the arrangement, it would be divided into the following parts.

Part I. A systematic course of about ninety hours, fairly concentrated in the later part of the second year, in which the technic of the ordinary simple routine tests is to be perfected, and a general understanding obtained of their relation to clinical diagnosis. We should not only teach the positive value, but also the negative value and limitations of laboratory findings.

The object of such a course is so that the student can start in, well prepared for the usual required laboratory examinations of the cases to which he may be assigned in his third year. The content should be routine urinalysis, hemoglobin, blood counting, appearance of cells, blood grouping, malarial parasites, stomach contents, feces for blood, parasites and ova, sputum and purulent exudates,, including spinal fluid, for cytology and pathogenic organisms.

Part II. A course of about ninety hours in the early part of the third year, extending over three or four months. This represents a study of important diseases in which the laboratory viewpoint will be thoroughly worked out, clinics being held upon selected cases, showing the indications for and limitations of certain tests. Students under supervision will carry out the more ordinary routine tests simultaneously, checking their work, and instructors with student assistance will demonstrate all other more complicated and possibly useful ones that may throw light on the clinical diagnosis, thereby presenting complete exhaustive laboratory studies of the important diseases.

Part III. During the later part of the third and the whole of the fourth year, under proper supervision of technic, and consultation in regard to in-

dications for and result of tests, the students carry out the ordinary laboratory examinations upon the cases to which they have been assigned in hospital and outpatient service. Time does not permit the average student to do more.

Part IV. Elective courses. To properly qualified students of the third and fourth years, elective courses covering more thoroughly the more complicated technical procedures should be offered. Only subjects of clinical value should be included.

The proper observation of the working out of the tests in concrete cases, with enough clinical material for comparison, is in our opinion the real criterion of a Class A course in clinical pathology.

In some schools it may be found advisable to give the whole of the systematic course in the second year; in others more hours may be given in the second than in the third year. Such changes could readily be made, depending upon the permissible allotted time and proper arrangement to other courses.

We believe, however, that there should be promoted a better standardization, so as to place more equitably the year given, hours required and content of course, in order that the status will be more definitely recognized and fixed in the medical curricula of all medical schools.

SUMMARY

There has been in the medical curriculum a need and consequent development of a course for the perfection of technic and interpretation of laboratory methods used in diagnosis. This is most frequently designated as Clinical Pathology, or Clinical Laboratory diagnosis.

From a survey of replies to questionnaires sent to teachers of clinical pathology, medicine and surgery, and from catalogues, the following information has been obtained:

1. Courses in histology, biochemistry, bacteriology, immunology, and part if not all, of general and special pathology, should be completed before clinical pathology is taken up.

2. The time at which the course is given by the majority of schools is the first part of the third year; next in number is the latter part of the second year; some give it in the latter part of the second and first part of the third, a few others throughout the whole third year. All schools advise a continuation of laboratory work, under supervision, on cases later assigned to the student

3. In hours, the courses vary in the different schools from 36 to 252 hours; about one-third from 120 to 252; one-third from 90 to 120, and one-third from 40 to 80. The average number of hours was 100. One hundred eighty hours was recommended by the Association of American Medical Colleges in 1921. Three-fourths are opposed to the daily concentrated course.

4. The course should cover more than the ordinary routine examinations that a general practitioner would be expected to use in his office, was the opinion of more than three-fourths of the replies of the clinical pathologist.

Most clinical teachers replied that only ordinary laboratory methods be taught together with the application of these and the more complicated methods.

5. There appears to be considerable difference of opinion as to what special subjects out of the ordinary should be included. Most frequently required are blood chemicals, Widals, and blood cultures; most frequently demonstrated are complement-fixation and colloidal gold and basal metabolic tests; most frequently elective are basal metabolic, blood chemicals, and serodiagnostic methods. Most of the subjects are given in their fundamental aspects in other departments, their clinical importance demonstrated in the laboratory of clinical pathology and at the bedside.

6. Two-thirds of the replies of the clinicians are of the opinion that the student depends too much upon the laboratory findings, and two-thirds of the clinical pathologists believe that he does not. There is a general opinion, where this fault exists, that some of the teachers in the preclinical subjects do not keep sufficiently in touch with the clinical side of medicine, also the fault of some of the clinicians, who do not have a clear understanding of the use and interpretation of certain tests. By some of the clinicians, it is thought that too great a proportion of time is devoted to the laboratory.

An outline of a course is presented showing place in curriculum, hours, and content which is believed to be suitable for schools in which there is, in close physical relationship, a teaching hospital.

A plea is extended for a better, more equitable standardization of the course and a more definitely fixed status of clinical pathology in the curricula of the medical schools.

CERVICAL SYMPATHECTOMY IN ANGINA PECTORIS*

With Report of a Case

By William H. Holmes, M.D.,† and S. W. Ranson, M.D.,‡ Chicago, Ill.

THE first attempt to relieve the pain of angina pectoris by removing portions of the left cervical sympathetic chain was made by Jonnesco[1] in 1916. Four years later when he presented his report, the patient had had no recurrence of pain. That this remarkable result did not attract more attention was probably due to the fact that Jonnesco had previously advocated cervical sympathectomy for other conditions such as hyperthyroidism, tic douloureux, epilepsy and glaucoma, and had claimed results which others subsequently were unable to obtain. The operation of cervical sympathectomy, therefore, was generally abandoned. Very recently, however, Coffey and Brown,[2] impressed by the report of Jonnesco's case of angina pectoris decided to sever the connections between the superior cervical sympathetic ganglion and the heart in a patient having frequent and severe anginal attacks. The operation was followed by complete relief from pain. Nine days later they performed the operation on a second patient with similar results. Their third and fourth patients were also relieved of pain but the fifth died six hours after operation. Brown,[3] one of the surgeons, advises us that at first they cut the main trunk of the left cervical sympathetic and the superior cardiac branch arising from the ganglion itself. Later on they removed the ganglion in order to be certain that all of its connections were cut. They have performed the operation twelve times, once on the right side in a case of dextral radiation. The attacks of substernal pain were stopped in every case but one, a syphilitic with aneurysm. In two cases a modified form of pain persisted in the region of the apex and left forearm.

The objective signs following removal of the ganglion are enophthalmos, myosis and pseudoptosis, narrowing of the palpebral aperture and absence of sweating and flushing on the affected side, constituting the syndrome of Horner. If the entire cervical sympathetic chain on one side is injured, the exact area in which sweating does not occur is bounded by a line which runs down the middle line of the head and neck and turns horizontally across the chest at the level of the third rib in front, and the spine of the scapula behind, including the whole of the upper limb.

In our own case, as in those of Coffey and Brown the paroxysmal pain of angina pectoris was relieved by removal of the superior sympathetic ganglion. The patient was a white woman, aged fifty-three years, who was

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admitted to Wesley Memorial Hospital on January 29, 1923, for treatment of cardiac failure. For about one year prior to the appearance of edema, dyspnea, and other signs of decompensation, she had vague pains in the precordium and left arm. During the first part of her hospital residence she did not complain of pain. Her first attack of typical angina followed the effort of getting into a wheel chair. Thereafter, pain recurred with increasing frequency and severity until the slightest exertion was sufficient to bring it on. Nitroglycerin and amyl nitrite gave almost immediate relief but the subsequent prostration was extreme. On June 12, 1923, the superior cervical sympathetic ganglion was removed by William E. Shackleton and saved for histologic

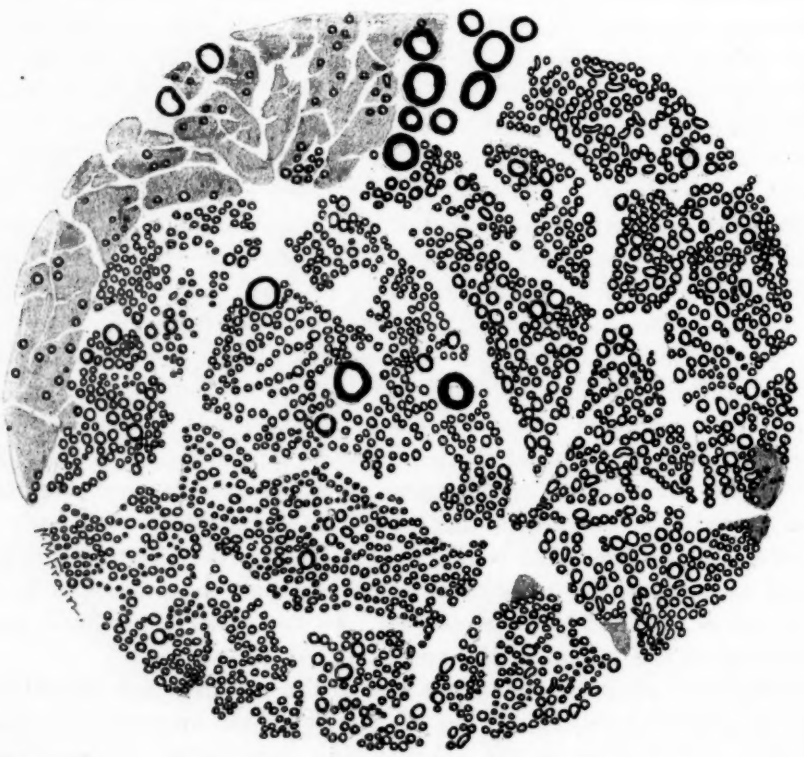

Fig. 1.

study. She was discharged from the hospital August 6, 1923, and soon thereafter was earning her living as a nurse in charge of two small children. At no time following the operation did she experience pain either in the thorax or left arm. Just a few days before the Christmas holidays she started on an automobile trip to Atlanta, Georgia. En route she became ill and was admitted to the Nashville General Hospital. A letter from the hospital dated December 29, 1923, states that she complained of severe abdominal pain, nausea, vomiting and of mild precordial pain just before her admission. The temperature, pulse, respiration, blood pressure, etc., were reported to be about normal. She recovered from this undiagnosed illness, was discharged from the hospital, and is now in Chicago. During the eleven and one-half months

she has remained under our observation, she has not had a single recurrence of thoracic or brachial pain of the anginal type.

Without entering into a lengthy discussion of the various theories which have been advanced in explanation of the symptomatology of angina pectoris it seems reasonable to assume that the structural changes are in themselves, insufficient to produce the paroxysmal symptoms observed in the ordinary case. In the extraordinary case in which the structural changes are extensive and of rapid development as in coronary thrombosis, pain is present in agonizing intensity but it is continuous, not paroxysmal. Allbutt believes the additional factor required to produce paroxysmal anginal pain is essentially a change in intraaortic pressure. Others think vascular spasm either of the aorta or coronary arteries is the cause of the pain.

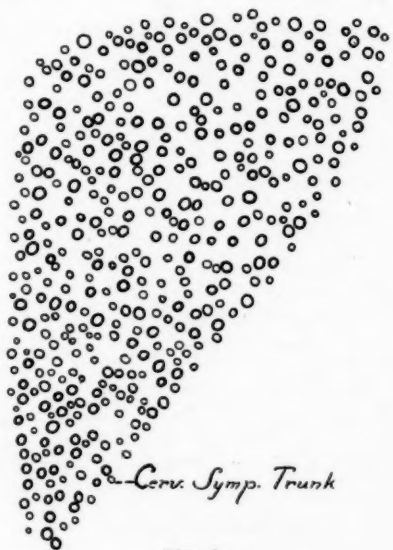

Fig. 2.

Surgical methods of treatment of angina pectoris have, heretofore, always been directed toward the relief of pain by severing the sensory fibers to the heart and aortic arch. This was accomplished by Jonnesco when he removed the lower cervical and first thoracic sympathetic ganglia, but at the same time he also destroyed efferent fibers to the aortic arch and coronary arteries. As shown by Coffey and Brown and confirmed by our own case the same beneficial results may be obtained simply by removal of the superior cervical ganglion, through which, according to the best information available, none of the cardiac sensory fibers pass. We would attribute the beneficial effect of high cervical sympathectomy to section of vasoconstrictor fibers, instead of to section of sensory fibers as is done by Coffey and Brown. It is important, therefore, to determine the course of the sensory fibers from the heart and the course of vasoconstrictor fibers going to the coronary arteries and aorta. The course of the cardiac sensory fibers is, we believe, fairly well established on the basis of histologic, physiologic and clinical evidence.

Histologically, it is possible to trace sensory fibers through the sympa-

thetic system because of their relatively large size. Edgeworth[4] has traced large sensory fibers to the cardiac plexus in the dog from the vagus nerve and from the white rami of the upper three thoracic nerves. Those from the white rami reached the cardiac plexus after passing through the stellate ganglion, the annulus of Vieussen, the inferior cervical ganglion and the nerve passing from that ganglion to the heart. One of us (S. W. R.)[5] by the study of serial sections of the sympathetic trunk in the cat has been able to confirm Edgeworth's findings. The large sensory fibers in the upper three thoracic white rami (of which the second is illustrated, Fig. 1) can be traced through the annulus of Vieussen and thence through the cardiac branches that spring from it, and from the inferior cervical ganglion. No large sensory fibers were found in the cervical sympathetic trunk going to the superior ganglion. The nerves removed from our case of angina on histologic examination showed no large sensory fibers in either the cervical sympathetic trunk or the superior

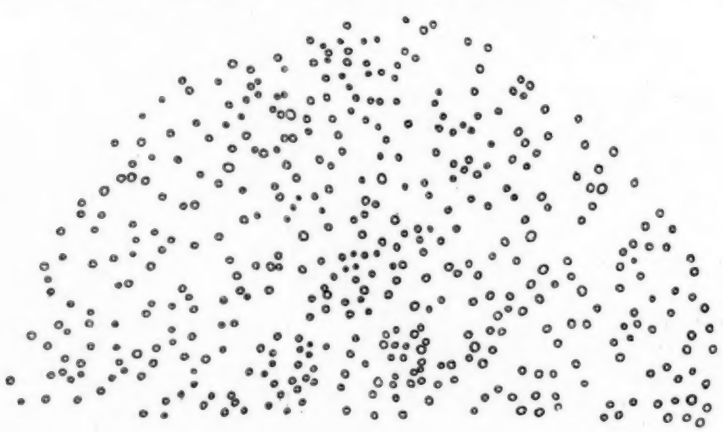

Fig. 3.

cardiac nerve which descends from the superior cervical ganglion to the cardiac plexus. (Figs. 2 and 3.) There is, therefore, no reason for assuming that the sensory pathways for the heart in man are essentially different from those in the cat and dog. The course of the sensory fibers from the heart as determined by histologic studies is shown in the diagram. The arrows indicate the direction of conduction and the dotted line a possible but highly improbable pathway. (Fig. 4.)

This conception of the sensory pathways is supported by physiologic and clinical evidence. Langley, using electrical stimulation, could find no evidence of sensory pathways in the cervical sympathetic trunk. Head has shown that the thoracic and brachial pain in angina pectoris is referred along the distribution of the three upper thoracic nerves of the left side. This demonstrates that this type of pain is mediated by the white rami of these three nerves. It excludes the possibility that it is mediated either through the vagus or through hypothetical connections of the superior cervical ganglion with the brain. In this connnection it will be remembered that Wencke-

bach in his address before this Society last year claimed to have stopped anginal attacks by cutting the depressor nerve. While this nerve is recognized as a distinct branch of the vagus in the rabbit, its fibers are incorporated with the vagus in man. Granting that it may have a separate course for a short distance as Odermatt[6] and some other European workers claim, it is scarcely conceivable that pain which is referred to the chest wall and left arm, should be relieved by section of any part of the vagus nerve. We cannot help wondering if in these operations the superior cardiac nerve which runs through the operative field has not been damaged. Nor can the relief

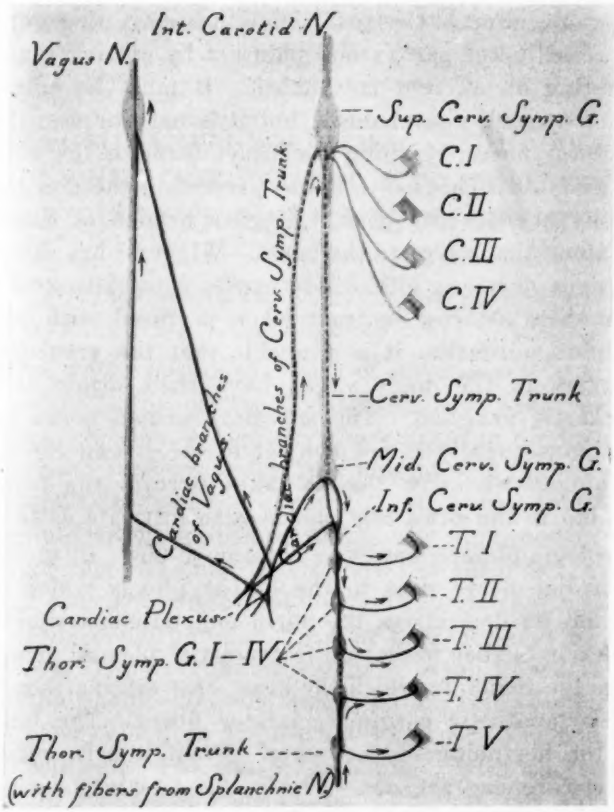

Fig. 4.

given by high cervical sympathectomy be due to the interruption of sensory paths through the thoracic white rami, because these nerves are not even exposed to operative trauma in removing the superior cervical ganglion. That the sensory pathways through the thoracic white rami are not destroyed by resection of the superior cervical ganglion is also indicated by the fact that this operation does not invariably stop referred pain of cardiac origin. The continued pain in Coffey and Brown's case of syphilitic aneurysm in spite of the removal of the superior sympathetic ganglion may have been due to direct pressure on the vertebrae or sternum, but this would not explain the presence of pain in the forearm in two of their uncomplicated cases of

angina. The persistence of pain in the forearm proves the presence of intact sensory pathways through thoracic white rami to the corresponding segments of the spinal cord.

That cervical sympathectomy does not relieve pain merely by interrupting sensory pathways is also indicated by the fact that in successful cases patients are often returned to a life of usefulness. The sense of constriction, the fear, the physical prostration and other evidences of angina pectoris were in our case relieved quite as much as was the pain. From being bedridden because of anginal attacks brought on both by effort and emotion, the patient, after the operation, was able to follow a gainful occupation requiring considerable effort. Cervical sympathectomy, therefore, apparently brings about a cessation of paroxysmal pain not by cutting sensory pathways but by interrupting an efferent mechanism. It must be admitted that we know nothing about such a mechanism, but it is not unreasonable to suppose that vasoconstrictor fibers, reaching the heart through the superior cardiac nerve, may be at fault. This nerve is not present in the dog but its place is perhaps taken by fibers which pass through a branch of communication to the vagus and along that nerve to the heart. Wiggers[7] has shown that stimulation of the vagus nerve on either side causes constriction of the coronary arteries. Since these arteries contract when perfused with physiologic salt solution containing adrenalin, it is probable that the vasoconstrictor fibers found by Wiggers in the dog's vagus have their origin in the superior cervical sympathetic ganglion. The superior cardiac nerve is not present in animals and consequently is not subject to direct experimental investigation. In the human we know that it takes part in the formation of the cardiac plexus but in the adult this plexus is so intricate as to defy analysis.

The embryologic picture, however, gives us a clue. In the human embryo the superior cardiac nerve goes to the bulbar plexus which surrounds the bulbus cordis and its derivatives the aorta and pulmonary artery while the middle and inferior cardiac nerves go to the atrial plexus. The atrial plexus, since it supplies the region in which the heart beat originates may be assumed to receive the sympathetic cardio-accelerator fibers. The bulbar plexus is situated near the beginning of the aorta. From it arise offshoots forming plexuses on the coronary arteries. These offshoots may be assumed to receive vasoconstrictor fibers which probably come through the superior cardiac nerve. Since the aorta is formed from the left side of the divided bulbus cordis it might be assumed that the vasoconstrictor fibers for it come from the left superior cardiac nerve. If future investigation should prove that this assumption is correct it would be possible to explain the effects of high left cervical sympathectomy as due to the interruption of the vasoconstrictor path to the aorta and coronary vessels.

The idea that the paroxysms of angina pectoris are due to structural change plus vascular spasm has long held a prominent place among the theories advanced in explanation of the symptoms of the disease. Not being able to account for the results obtained by section of the superior cardiac nerve on the basis that it contains sensory pathways we advance the hy-

pothesis that this nerve contains vasoconstrictor fibers for the aorta and coronary arteries and that section prevents spasmodic vasoconstriction and, therefore, stops paroxysmal pain, but is without effect on pain caused entirely by structural changes.

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ON THE ALLEGED SYNERGISM OF MAGNESIUM SULPHATE AND MORPHINE WHEN INJECTED PRIOR TO THE INDUCTION OF ANESTHESIA BY THE ETHER-OIL COLONIC METHOD*

By Harry Beckman, M.D., Milwaukee, Wis.

IN 1921 Gwathmey,[1] reviewing the work of Meltzer and Auer and others by which the anesthetic properties of magnesium sulphate were established, concluded "that magnesium sulphate is a safe agent in producing anesthesia" and that "when used synergistically, it is absolutely harmless and one of the most effective agents of which we know." He states that the synergistic effect of magnesium sulphate upon morphine was suggested to him by Pellini and that working together they determined the effect of this combination, as follows: "A sufficient number of animal experiments was conducted to prove that ⅛ grain of morphine in 2 c.c. of a 25 per cent solution of chemically pure and sterilized magnesium sulphate, given hypodermically and repeated twice at half-hour intervals, analgized an animal sufficiently for the full force of an artery clamp to be placed anywhere on the skin without being noticed by the subject. The controlled animal, with the same dosage of morphine given in plain water hypodermically, was not analgized to anything like this degree." This is adduced as evidence of the effectiveness of the combination, nothing being brought forward to prove the "absolutely harmless" nature of the same—indeed, no mention is made of the relative state of depression of these animals either during or following the experiments. As further evidence in favor of the combination, the statement is made that "at the Presbyterian Hospital it has been definitely determined that the addition of a small amount of magnesium sulphate to the usual hypodermic of morphine increases the value of the hypodermic from 50 to 100 per cent."

Gwathmey therefore proposed the conversion of colonic anesthesia, as previously developed by him, into synergistic analgesia by making use of the

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synergistic effects of magnesium sulphate and morphine when the two drugs are injected hypodermically prior to the introduction into the colon of the ether-oil mixture.

Since the appearance of the paper referred to, Weston and Howard[2] have reported the giving of from two to six c.c. of a 50 per cent magnesium sulphate sterile solution, subcutaneously and intramuscularly, in more than 1000 instances in the insane in states of more or less excitement and insomnia. In 82.7 per cent of the cases the sedative action was prompt, the patient becoming quiet after fifteen to thirty minutes and sleeping from five to seven hours. In a few instances a patient became quiet but did not sleep. "The effect persisted for from five to ten hours." They have also used the salt in a number of persons, not insane, but suffering from severe pain, with very gratifying results. They failed to find any evidence in favor of the alleged synergism of magnesium sulphate and morphine, for they found "that in cases not quieted by six to 10 c.c. of 50 per cent magnesium sulphate solution, morphine was also of no value except in heroic doses, and that when the magnesium sulphate alone failed to give the relief it was of no effect when used in combination with ¼ grain of morphine."

Recently Gwathmey, Donovan, O'Reagan, and Cowan[3] have published a preliminary report of clinical experiments having for their object the production of painless childbirth by synergistic methods, one of the drugs advocated being magnesium sulphate. They state that with the synergistic method they attempt to secure the relaxation with the magnesium sulphate as well as using it for its power of prolonging the effect of the morphine. In this report they are concerned chiefly with the relief of pain and the condition of the baby.

In view of the paucity of experimental data upon which to base a belief in the synergism of magnesium sulphate and morphine, the writer has undertaken a study of the question by performing a definite number of carefully controlled experiments.

The work has been projected upon the following schema, dogs being used as subjects in all cases:

(*a*) A series of trials in which morphine alone is to be injected before inducing anesthesia by the ether-oil colonic method; the object here is the determination of the exact amount of ether which can always be counted upon to induce and maintain surgical anesthesia.

(*b*) A series of trials in which both magnesium sulphate and morphine are to be injected before inducing anesthesia by the ether-oil colonic method, using an amount of ether less than that determined in (a) to be always effective. The object here is twofold: First, to determine whether the amount of ether can be radically reduced because of the synergistic effect of the magnesium sulphate upon the morphine—in other words whether ether-oil anesthesia can be converted into ether-oil analgesia, due to the action of the magnesium sulphate. Second, to observe any deleterious symptoms which might arise subsequently to the injection of the magnesium sulphate, and which might rightly be attributed to the same.

(*c*) A series of trials in which calcium salts are to be injected at such time, and in such manner, as best to overcome any deleterious effects of the magnesium which might be observed in (b). The object here would be to lessen the toxicity of the magnesium without decreasing too much its synergistic effect upon the morphine.

Series (a) and (b) of these observations will be reported upon in this paper.

SERIES (A)

Method.—Dogs, having been starved for eighteen hours, were injected subcutaneously in the side with an aqueous solution of 0.01 gm. morphine sulphate per kilo body weight. After one hour they were tied to a dog-board upon their left side; a No. 29 soft rubber catheter was lubricated and introduced slowly into the rectum and colon for an average distance of seven inches, and the ether-oil mixture allowed to flow in during four minutes. Gwathmey[4] having suggested 75 per cent ether and 25 per cent olive oil, a mixture in these proportions was used in all cases, with the following results:

(1) One ounce of the mixture per 40 lbs. of dog was ineffective in inducing anesthesia.

(2) One ounce of the mixture per 30 lbs of dog was little if any more effective.

(3) One ounce of the mixture per 20 pounds of dog was in all cases effective in inducing and maintaining surgical anesthesia. Ether was usually detected on the breath before the last of it had been introduced into the colon, anesthesia being complete in an average time of thirteen minutes. There were no failures. In a few cases there was some slight loss due to bubbling of gas up through the tube, or around it at the anus; but estimate of the amount lost was made and then that amount added. Complete surgical anesthesia lasted from one and a quarter to one and a half hours; in two cases, at the first signs of returning consciousness, another full dose of the ether-oil mixture (one ounce per 20 lbs.) was introduced and anesthesia maintained for an additional hour and a half. The usual test for complete anesthesia was the ability to make an abdominal incision through all the layers of the skin in a dog having complete muscular relaxation. However, in order to be doubly sure, in three cases (selected at random) the abdomen was completely laid open and rough manipulative procedures were performed upon the gut. Muscular relaxation was always complete, though the partial retention of reflexes was always noted; in two cases dogs responded to a whistle by partially raising the head while major surgery was being done on the abdomen.

Careful observation of the respirations and pulse were made in all cases, and the absence of salivation and of any movements of the body in the second (excitement) stage remarked in all cases.

A single attempt to use the method without preliminary morphine failed; the dog fought fiercely, and the very small amount of the mixture which was successfully introduced served only to produce a violent excitement stage which lasted for some time after the dog was released.

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In this series eight dogs were used, some of them being anesthetized as often as five times at intervals of one week. Altogether the method was used twenty-four times. A protocol, selected at random from the series, is here given:

Dog B.—March 3. Male, 14 kilos, estimated age 1 yr. Condition good, 3rd anesthetic.
12:00 M. —Morph. Sulph. subcut., right side, 0.14 gm. (3.5 c.c. of a 4 per cent solution.)
1:08 P.M.—Placed on board, tied down on left side.
Resisted pricking of skin of abdomen with knife.
Respiration 18, pulse 84.
1:14 to 1:21 P.M.—Rectal tube introduced (very little resistance) and 47 c.c. of ether-oil (75% E, 25% O) introduced (1 oz. per 20 lbs.).
1:26 P.M.—Dog cannot be aroused but eyes are open.
Lid reflex undiminished.
1:30 P.M.—Respiration 20, pulse 104.
Untied and placed on back. Muscular relaxation complete, eyes half closed.
Lid reflex present but diminished.
1:35 P.M.—Incision made down to peritoneum. Anesthesia perfect.
Three interrupted sutures, painted with iodine and alcohol;
Respiration 24, pulse 116.
1:40 P.M.—Anesthesia continues. Respiration 24, pulse 120.
1:45 P.M.—Anesthesia continues. Respiration 24, pulse 120.
1:50 P.M.—Anesthesia continues. Respiration 26, pulse 116.
2:00 P.M.—Anesthesia continues. Respiration 26, pulse 122.
2:10 P.M.—Anesthesia continues. Respiration 28, pulse 122.
2:20 P.M.—Anesthesia continues. Respiration 28, pulse 120.
Full force of hemostat on belly wall; no response.
2:25 P.M.—Introduced small catheter beside the other and began washing out.
2:31 P.M.—Washing out completed, 500 c.c. tap water. Returning water oily with slight odor of ether.
2:36 P.M.—Moving head about and pawing with front legs.
2:52 P.M.—Rolled himself off the dog board.
3:02 P.M.—Dog returned to kennel; moves about on legs groggily, selecting place to lie down; lies down in natural position.
4:00 P.M.—Sleeping but quite easily aroused.
March 4.
9:00 A.M.—Dog completely recovered.

In none of the dogs has there been any symptoms of colitis, or of even the very slightest rectal irritation. One of the dogs fell sick after having been anesthetized (colonic) five times at intervals of one week. Symptoms: malaise, weakness of front legs, temperature 103.2° F. This dog was then given the usual dose of morphine but anesthetized by the inhalation method, 25 c.c. of ether being required by the open drop method to establish the surgical stage. The animal was used for routine laboratory work and at the end of two and one-half hours, when killed, a total of 161 c.c. of ether had been used; the amount required in the colonic method in this length of time would have been 86 c.c. (115 c.c. of the mixture). The colon and rectum were carefully examined and no evidence whatever of inflammation therein could be found. This dog had several wounds upon the abdomen and probably died from septicemia; the other organs were not examined.

In the interval of one hour, or a little more, between the injection of the morphine and of the ether-oil mixture, the dogs were allowed their freedom

in a large space. During this time they defecated and urinated several times, but vomited only rarely. They feebly resisted catching, and the weakness of the hind legs, typical of morphine, was observed in all cases. When placed upon the anesthetizing table they showed no signs of respiratory embarrassment, though the amplitude of the respirations was perhaps slightly less than in the normal and the rate a little slower. In all cases they were able to stand, though somewhat groggily, one-half hour after the ether-oil mixture had been washed out of the colon, and when removed to the kennels at this time they would always move about and select their own place for lying down. They were usually removed to the kennels between four and five o'clock in the evening, were always found to be completely recovered at nine in the morning, and remained in a perfectly normal state thereafter.

SERIES (B)

Method.—Dogs, having been starved for eighteen hours, were injected subcutaneously in the side with an aqueous solution of 0.01 gm. morphine sulphate per kilo body weight (same amount as in Series (a)), and at the same time, either in that or the other side, magnesium sulphate was injected, 2 c.c. of a 50 per cent solution being given in all cases. The site of the magnesium sulphate injection was massaged for about one-half minute. After one hour they were tied to the dog board and the catheter introduced, but the amount of the ether-oil was reduced to one ounce per 40 lbs., an amount which had always been ineffective in inducing anesthesia when morphine alone had been previously injected.

With the same criteria for surgical anesthesia as in Series (a), it cannot be said that anesthesia was ever successfully induced in this magnesium sulphate series. The dogs were brought to the table in apparent distress in all cases; they were whining; the rate of the respirations was not reduced more noticeably than when morphine alone was used, but the respirations were jerky and there was almost an expiratory grunt; and in most cases there was a quite profuse salivation. A number of these dogs have pawed at their nose, a symptom which is taken by many observers to be a sign of respiratory embarrassment. The jerky respirations have persisted throughout the attempt to induce anesthesia, and several times the moaning has persisted also. Though the dogs have usually seemed to lose consciousness, they were never completely relaxed, and always resisted deep cutting. They fail to return rapidly to consciousness after being washed out and are much depressed for usually two days after.

In this series five dogs were used, some of them as often as four times at intervals of one week. All of the dogs had previously been successfully anesthetized in Series (a). Altogether the method was used twelve times. A protocol, selected at random from the series, is here given:

Dog E.—April 7. Male, 9 kilos, estimated age 1½ yr. Condition good. Two anesthetics in Series (a).

12:00 M. .—Morph. Sulph. subcut., right side, 0.09 gm. (2.25 c.c. of a 4 per cent solution). Mag. Sulph. subcut. right side, 1.0 gm. (2 c.c. of a 50 per cent solution). Massaged ½ min.

1:04 P.M.—Placed on board, tied down on one side. Dog moaning constantly. Respiration 24, jerky. Pulse 108. Marked salivation.

1:08 P.M. to 1:16 P.M.—Tube inserted, 16 c.c. of ether-oil (75 per cent E., 25 per cent O) introduced. (1 oz. per 40 lbs.).

1:20 P.M.—Respiration 26, jerky. Pulse 110.

1:24 P.M.—Moaning ceased, resp. continued jerky.

1:30 P.M.—Dog placed on back but rolls himself on to left side again.

1:35 P.M.—Resp. 26, jerky. Pulse 110.

1:40 P.M.—Dog placed on back and remains there but is not completely relaxed. Attempt to incise abdomen is resisted by movements of legs and moaning is recommenced.

1:50 P.M.—Respiration 28, jerky. Pulse 118. Stops moaning. Attempt to incise unsuccessful as before; moaning does not recommence.

2:02 P.M.—Attempt to incise resisted. No moaning.

2:05 P.M.—Catheter introduced and washing out begun.

2:12 P.M.—Washing out completed, 500 c.c. tap water. Returning water oily with slight odor of ether.

2:30 P.M.—Has made several voluntary movements of legs but is still lying on back on the board.

2:50 P.M.—Removed to kennel. Very limp, lies in unnatural position in which placed. Resp. continues jerky.

4:00 P.M.—Dog has changed position but very little. Awake and moaning. Respiration continues jerky.

April 8 and 9. Dog appears sick. Lies on side, or wanders around kennel as if in a stupor. Moans for long periods. On April 9, at 4 P.M., expirations still partake somewhat of their former jerky character.

April 10. Dog appears normal.

A typical picture in this series on the first, and to a less extent on the second day after the injection of the magnesium sulphate, is of a very sick looking dog lying flat on the side with legs extended, moaning, and with jerky expirations; this will alternate with periods of standing in a corner with drooped head, with moaning, and jerky expirations, stupid expression, and an occasional lurching forward to keep the body from falling. The salivation persists only for a few hours after injection of the salt. A large abscess has appeared at the site of the magnesium injection in five of the twelve cases, whereas no abscesses have occurred from the morphine injections in either of the series. The pus evacuated has not been cultured, but is odorless. The solutions used have not been sterilized.

In two cases, outside this series, 4 c.c. of the magnesium sulphate were given, the other conditions being the same as when only 2 c.c. were given. In one of these cases the anesthesia was complete, which was proved by performing mutilating operations in the abdomen; the dog was of course killed at the end of the experiment. In the other case the anesthesia was never perfect, and the depression after the dog was washed out was so great that calcium chloride was given to save the dog; though as the calcium chloride was injected subcutaneously (4.5 c.c. of a 50 per cent solution), it is not certain that it was an effective agent in promoting this recovery.

DISCUSSION

Series (a).—So far as a parallel may be drawn between dog and human work, all the advantages claimed by Gwathmey[5] for ether-oil colonic anesthesia are amply borne out by these experiments. These advantages can perhaps best be pointed out by making use of Gwathmey's original headings:

"Safety."—While the induction of third stage anesthesia with ether in an average time of thirteen minutes, by placing within the body at one time the entire amount of ether to be used, may appear somewhat dangerously precipitate, it is in fact only apparently so. The rate at which ether separates from ether-oil mixtures *in vitro* was shown by Baskerville[6] in 1915 to be a definite and fairly fixed one. This definite rate of separation must apply also under the conditions obtaining *in vivo;* for were it possible for all, or a large part, of this ether to become available for absorption into the circulation at any one time, it should be expected that at least some cases of too profound anesthesia, if not actual deaths, would have been encountered in the series. No such cases occurred; but on the other hand, in all instances a definite quantity of ether per pound body weight produced a satisfactory, but not too deep anesthesia, which lasted for a predictable length of time, after which the animal quickly regained consciousness. This is taken as evidence that only a small portion of the total amount of ether placed in the colon is available to the circulation in a unit of time. The partial retension of reflexes (the lid reflex has never entirely disappeared) must be taken as further evidence for safety; indeed some of the animals seemed not entirely to have lost consciousness, for in two cases feeble response was made to a whistle while major surgery was being performed upon the abdomen.

"Comfort."—The animal is not subjected to the indignity of being forcibly held while the objectionable cone is placed over the muzzle, nor does he make even the slightest struggle during the second stage.

"Control."—When the correct amount of the mixture is administered per pound body weight, the method is absolutely controllable in both directions: the anesthesia may be terminated at any time by washing out the colon with tap water, with a rapid return of consciousness; the state may be prolonged for any desired length of time by adding, for every additional hour of anesthesia required, one ounce of the mixture per 20 lbs. body weight. If, at any time, the anesthesia seems too light, a cloth may be placed over the muzzle, thus compelling a partial rebreathing of the fumes, with an appreciable deepening of the state.

"Efficiency."—Whenever one ounce of the mixture per 20 lbs. is used perfect anesthesia results. The actual amount of ether used—22.5 c.c., 75 per cent of the mixture—for inducing and maintaining surgical anesthesia is much smaller than required in any other method available to us for safe work in the laboratory.

"Simplicity."—The apparatus required is one No. 29 soft rubber catheter, one No. 13 same, a spring or screw clamp for each, two small funnels, and a receptacle to catch the oily water when washing out the colon. One introduces

the large catheter, which has previously been filled with the mixture so that it just shows in the attached funnel, for about 7 inches, and then slowly (during 4 minutes) pours in the measured quantity of the ether-oil, closing the clamp when the fluid just shows again in the funnel. The catheter is allowed to remain in place; the writer has usually tied it to the tail with a bit of string. At the conclusion of the experiment, the small catheter is introduced beside the larger one, the latter withdrawn somewhat, and the animal is washed out, introducing the water through the small catheter. The articles are always clean and never objectionable to use, an advantage which cannot always be claimed for the foul, saliva-soaked ether cones commonly employed in laboratories.

"After-effects."—Symptoms of colitis have never been encountered. Recovery has always been prompt and lasting. A measure of analgesia seems to persist for some time, as the animals in this series have seemed startlingly unaware of their wounds several hours after regaining consciousness.

Series (b).—To the first of the questions investigated in this series, namely, does magnesium sulphate exert a synergistic effect upon morphine sulphate when injected prior to the induction of ether-oil colonic anesthesia, it is felt that a positive answer cannot be given at this time. I certainly failed to obtain any evidence in favor of this synergism when I gave the amount of magnesium sulphate (2 c.c. of a 50 per cent solution) which is said always to increase the value of the morphine from 50 to 100 per cent. However, I feel that the reduction in the amount of ether attempted may have been too radical, since Gwathmey,[1] in speaking of the reduction of the amount of ether to one-half in the human, states that 2 c.c. of a 25 per cent magnesium sulphate solution was given three times at intervals of one-half hour, the total amount of the salt injected thus being one-third more than was used by me. Therefore, I feel that the only statement warranted at this time is that under the conditions obtaining in these experiments no evidence in favor of the alleged synergism was obtained.

To the second of the questions investigated, namely, are there any deleterious symptoms arising subsequently to the injection of the magnesium sulphate which may rightly be attributed to the same, a positive answer can be given. In all cases in which the salt was injected in these experiments such symptoms did arise. I believe that a careful study of the classical experiments by which the anesthetic properties of magnesium sulphate were established would lead us to expect such symptoms, even when such small doses are used as those advocated clinically and used in these experiments. 1. Meltzer and Auer[7] showed conclusively (working with rabbits) that magnesium salts in intravenous injections are very toxic, even in small doses; the effect being upon the respiration, which becomes completely inhibited. 2. Intraspinally in monkeys,[8] magnesium sulphate caused the death of two of the six animals. They also state here that the relaxing effects may last twenty-four hours or longer. 3. Subcutaneously in rabbits,[9] 8 c.c. of a 25 per cent solution produced anesthesia from which the animals recovered; no recorded observations of their condition on subsequent days. Twelve c.c. of the same

solution was fatal. 4. Subcutaneously in cats,[9] the safe range was quite narrow: 0.8 gm. of the salt per kilo was unsatisfactory, 0.9 gm. produced anesthesia with recovery (no subsequent observations recorded), 1.0 gm. was fatal. 5. Subcutaneously in dogs,[9] 2.0 gm. of the salt per kilo, or a little more, was required to produce anesthesia; but the anesthesia would come on slowly, gradually become more profound, and finally lead to death. No animal survived, though they were all in a satisfactory state of anesthesia for some time before the terminal depression. When the injection was given intramuscularly, or part intramuscularly and part subcutaneously, somewhat more satisfactory results were obtained in that some of the animals survived. A state of depression on subsequent days is not remarked, except that in one case cited, the dog had lost nearly 1 kilo in weight after five days. 6. Subcutaneously in guinea pigs,[9] doses between 1.0 and 1.25 gm. per kilo were effective with recovery; 1.8 gm. per kilo caused rapid death. 7. Subcutaneously in white rats,[9] the dose required to produce anesthesia—1.75 gm. per kilo—resulted in death half an hour after injection in three of the five cases.

Justification for the use of this highly toxic salt subcutaneously in the human is found, so far as the writer can discover, in the following reasoning: the fatal dose for the rabbit being x, the fatal dose for a man weighing y times as much as the rabbit, would be xy. This dose being determined, a small fraction of it is then injected subcutaneously in man as an "absolutely harmless" dose. This small dose—2 c.c. of a 50 per cent solution—when injected with morphine, prior to induction of ether-oil colonic anesthesia in dogs, has now been determined to be provocative of symptoms which, if not absolutely dangerous, are certainly highly undesirable.

CONCLUSIONS

1. One ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 20 lbs. body weight, introduced into the colon of dogs one hour after the subcutaneous injection of ¼ c.c. of a 4 per cent solution of morphine sulphate per kilo body weight, is effective in inducing safe and very satisfactory surgical anesthesia and in maintaining same for a little more than one hour.

2. One ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 40 lbs. body weight, introduced into the colon of dogs one hour after the subcutaneous injection of ¼ c.c. of a 4 per cent solution of morphine sulphate per kilo body weight and 2 c.c. of a 50 per cent solution of magnesium sulphate, is not effective in inducing satisfactory surgical anesthesia.

3. The subcutaneous injection of 2 c.c. of a 50 per cent solution of magnesium sulphate at the same time that ¼ c.c. of a 4 per cent morphine sulphate solution per kilo body weight is injected in dogs, when these injections are followed after one hour by the introduction into the colon of one ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 40 lbs. body weight, gives rise to undesirable respiratory symptoms and a general depression, which usually lasts two days. These symptoms are directly referable

to the magnesium sulphate, because they do not arise when the salt is omitted, other conditions being the same.

These studies will be continued. The doses of morphine sulphate, magnesium sulphate and ether will be shifted about in many different combinations in an attempt to find some evidence in favor of the alleged synergism of magnesium sulphate and morphine. Calcium will also be used in an attempt to overcome the undesirable effects of the magnesium sulphate without lessening any synergistic effect it may be found to have upon morphine.

I wish to extend my hearty thanks to James T. Gwathmey, whose suggestions have been of the greatest help in planning these experiments.

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CHEMICAL BLOOD ANALYSIS IN ASTHMA, HAY-FEVER AND ALLIED CONDITIONS*

By Maximilian A. Ramirez, M.D., A. V. St. George, M.D., and R. L. Moses, A.B., New York

THE study of the various immunologic phenomena underlying anaphylaxis and allergy have been extensively investigated and reported; but it is only within very recent times that attention has been focused upon metabolic changes occurring in these conditions. Most of these studies, however, have been confined to animals. Thus Hisanobu noted increases in the nonprotein nitrogen and urea in the blood obtained from guinea pigs following anaphylactic shock. Hirsch and Williams found a diminished alkalinity of the blood in guinea pigs while in anaphylactic shock. In some of these animals the reaction was of such a magnitude as to be incompatible with life. A disturbance in the sugar metabolism, following the injection of protein and peptone, was noted by both Kuryama and Major and subsequently, Pentamelli demonstrated a marked disturbance in the nitrogen metabolism in rabbits following the injection of horse serum and milk respectively. Rackemann, Longcope and Peters, in a series of ten postpneumonic serum disease sufferers, noted a marked, transient chloride and water retention.

There is evidence accumulating that alterations in the physicochemical status of cellular activity is a controlling factor in some immunologic phenomena. Whether this is due to merely an altered hydrogen-ion concentration, a colloidal cellular change, or an increase or diminution in protoplasmic irritability are problems which require solution. It is with the object of contributing to the literature on this subject that we are presenting a series of chemical findings obtained during the routine examination and treatment of forty patients in our clinic.

Unfortunately we have only recently commenced the determination of the mineral constituents of the blood and hence have not as yet collected a large enough series for publication. Nevertheless, especially the calcium content of the blood in true bronchial asthma and in bronchospasm, promises to be of interest.

The nonprotein nitrogen sugar and plasma chlorides were determined on all patients when first seen at the clinic and again at the conclusion of treatment.† While some investigators might deem uric acid and creatinine determinations of value, we have not obtained any figures permitting of interpretation and hence we have discarded them. The nonprotein nitrogen and

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†It must be borne in mind that the blood was not taken immediately after the last injection or treatment, but usually several days or even a week later.

TABLE I

	NAME	SEX	AGE	DISEASE	BEFORE TREATMENT			AFTER TREATMENT			KIND OF TREATMENT	DURATION OF TREATMENT	RESULTS OF TREATMENT
					NPN	SUGAR	CHLORIDES	NPN	SUGAR	CHLORIDES			
					MG. PER 100 C.C.								
1.	J.A.	M	37	Asthma	36	102	640	34	110	510	Vaccine	16 months	improved
2.	C.M.	F	29	Urticaria	42	100	580	40	100	550	Food protein and diet	10 "	cured
3.	H.V.S.	M	64	Asthma	52	105	580	54	110	550	Horse dandruff	8 "	cured
4.	C.S.H.	M	39	Asthma	48	90	510	45	100	500	Vaccine	12 "	improved
5.	M.G.	M	48	Asthma	40	100	580	40	95	500	Vaccine	8 "	improved
6.	A.W.	M	36	Asthma	32	110	570	35	100	480	Vaccine	10 "	improved
7.	J.W.	F	28	Urticaria	36	100	590	35	100	540	Food protein and diet	6 "	not improved
8.	S.M.E.	M	29	Urticaria	40	105	540	40	100	500	Food protein and diet	4 "	improved
9.	J.H.H.	M	14	Hay-fever	28	100	520	34	95	500	Pollens	4 "	cured
10.	M.W.	M	26	Hay-fever	36	100	550	35	100	550	Vaccine and pollens	18 "	improved
11.	A.T.	F	28	Asthma	38	95	680	40	100	575	Vaccine	14 "	not improved
12.	C.R.S.	M	28	Urticaria	36	100	590	35	100	500	Food protein and diet	6 "	improved
13.	H.B.	F	29	Hay-fever	34	92	600	32	100	550	Pollens	4 "	improved
14.	M.K.	M	32	Asthma and Hay-fever	38	105	540	35	100	490	Pollens and Vaccine	18 "	cured
15.	H.O'B.	M	34	Asthma	40	110	550	40	100	500	Vaccine	13 "	improved
16.	K.S.	M	46	Urticaria	42	100	610	45	95	530	Food protein and diet	6 "	improved
17.	J.McM.	M	42	Hay-fever	39	105	550	40	100	540	Pollens	4 "	improved
18.	W.J.	M	26	Asthma	40	100	640	40	92	600	Vaccine	11 "	not improved
19.	E.M.	F	39	Eczema	32	105	590	30	100	510	Diet only	6 "	cured

TABLE I.—(Cont'd.)

	NAME	SEX	AGE	DISEASE	BEFORE TREATMENT			AFTER TREATMENT			KIND OF TREATMENT	DURATION OF TREATMENT	RESULTS OF TREATMENT
					NPN	SUGAR	CHLORIDES	NPN	SUGAR	CHLORIDES			
					MG. PER 100 C.C.								
20.	J.J.	M	28	Eczema	30	98	680	30	100	660	Diet only	8 months	cured
21.	H.P.	F	32	Eczema	38	100	650	38	100	660	Diet only	3 "	not improved
22.	J.M.	F	27	Eczema	35	110	650	40	100	600	Diet only	8 "	improved
23.	K.	F	38	Asthma	40	80	668	45	80	608	Horse serum	14 "	improved
24.	A.	M	24	Urticaria	80	182	650	49	122	563	Diet only	12 "	improved
25.	T.	M	51	Hay-fever	40	84	600	35	102	640	Pollens	2 "	improved
26.	S.	M	57	Asthma	39	96	640	37	87	588	Vaccine	12 "	not improved
27.	H.	F	38	Asthma	56.3	108	635	44	90	664	Horse dander	6 "	improved
28.	F.	F	28	Asthma	38	90	611	40	88	619	Vaccine	3 "	improved
29.	E.K.	F	42	Hay-fever	47	82	567	38	108	612	Pollens	2 "	improved
30.	R.E.	F	58	Asthma	35	80	625	38	82	603	Vaccine	8 "	improved
31.	L.C.	M	16	Hay-fever	50	94	662	47	85	588	Pollens	6 weeks	improved
32.	D.S.	M	21	Urticaria	33	80	631	38	100	588	Diet and Peptone injection	3 months	cured
33.	F.J.	M	16	Asthma and Hay-fever	32	92	625	33	85	625	Pollens	9 "	not improved
34.	M.H.	F	48	Urticaria and Asthma	34	116	625	39	80	625	Diet and Peptone injection	6 "	not improved
35.	C.C.	F	32	Hay-fever	41	90	650	50	90	618	Pollens	2 "	improved
36.	F.H.	M	29	Hay-fever	43	72	650	36	116	588	Pollens and Horse serum	3 "	improved
37.	L.H.	M	34	Urticaria	35	90	644	32	80	619	Diet and Peptone	4 "	cured
38.	A.S.	M	39	Asthma	40	92	650	40	90	637	Vaccine	6 "	improved
39.	E.H.	M	51	Asthma	40	83	675	32	80	613	Vaccine	6 "	improved
40.	T.B.	M	42	Hay-fever	42	76	650	35	82	600	Pollens	2 "	improved

sugar determinations were made in accordance with Folin's technic; the plasma chlorides according to the method of Friend, as modified by Hearn.*

As the standard average of these three constituents we have taken the following figures to represent the limits of normal.

Nonprotein nitrogen	25- 40 mg. per 100 c.c.
Sugar	80-125 mg. per 100 c.c.
Plasma Chlorides	550-600 mg. per 100 c.c.

These case records were selected, not on the basis of chemical reports, but on the clinical results—clinical cures—which were obtained. The patients have been free from symptoms at least six months and at least half of them were free of symptoms twelve to eighteen months from the cessation of treatment. The result of treatment was indicated as cured, improved and not improved.

Conclusions.—Nonprotein nitrogen, sugar and plasma chlorides were determined upon forty patients with asthma, hay-fever, and allied conditions. Determinations were made before instituting treatment and after completion of the same.

The resulting figures suggest that in asthma, urticaria, hay-fever, etc., there is a relative high plasma chloride content of the blood and to a less extent in the sugar and nonprotein nitrogen; the latter two especially in the nonallergic type of asthma cases with a long standing associated bronchitis.

It is impossible to state definitely that treatment caused a marked change in the chemical constituents of the blood, though a glance at the tables shows some diminution in the constituents after treatment.

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*The modification consists merely in the substitution of a standard solution of silver nitrate, of which 1 c.c. is equivalent to 1 mg. of sodium chloride, for the normal solution used by Friend.

A NOTE ON THE USE OF HEPARIN IN BLOOD TRANSFUSION*

By Edward C. Mason, M.D., Ph.D., Detroit, Mich.

INTRODUCTION

THE observations of Howell and Holt, which have been subsequently confirmed by the author, would lead one to conclude that heparin is an anticoagulant of first choice, it having no undesirable side reactions. Therefore in a previous study, based upon the results obtained from animals, it was recommended that heparin be considered as a clinical agent to inhibit intravascular coagulation. Before such clinical application could be made it was necessary to (1) develop a sterile product, (2) determine the toxicity for the human, (3) determine the dose necessary to give the desired delay in coagulation and (4) the length of duration of its action.

PROCEDURE

Sterilization.—The problem of sterilization of such a stable substance appeared to be quite a simple matter. However, in reality some difficulty was experienced in developing a sterile product without loss of potency. For clinical use it was recommended that heparin be put up in 1 c.c. ampules each containing 100 mg., but in the course of sterilization it was found more convenient to use 2 c.c. of solvent. Of the various methods of sterilization which have been developed, the choice for the present work has been to use 100 mg. of heparin dissolved in 2 c.c. of physiologic salt solution saturated with boric acid and 0.5 per cent chloretone.

The Determination of the Toxicity for the Human.—Prior to the use of the ampules in the human they were tested on several animals. Conditions were maintained analogous to those met with in transfusions except that the animals were either under ether or the effects of morphine. The apparatus used was that designed by Hartman. Such an apparatus furnishes a complete mixture of blood and anticoagulant soon after the blood leaves the vessel. The contents of the ampules were diluted with sterile physiologic saline to desired volume within the inner chamber of the apparatus.

The pharmacologic action of such preparations appeared to be quite analogous to that of the untreated heparin, there being no marked change in pulse, rate or volume, nor was there any noticeable change in respiration. For example: 150 c.c. of blood was drawn from a dog weighing 11.8 kilos, and the heparin solution was added at a constant rate. The total amount of heparin added was 32 milligrams which gave a concentration of approximately 1 milligram of heparin to 5 c.c. of blood. After ten minutes the blood was injected into the same animal and observations made. The animal appeared

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perfectly normal after the operation, and at no time subsequently were there any symptoms suggesting discomfort. The dose in this case was 1.23 milligrams per pound of body weight.

After seventeen days the animal just described was used again but the procedure was reversed, the anticoagulant being injected into the circulation before the blood was withdrawn. The dose of heparin selected was 200 milligrams on the assumption that an animal weighing 11.8 kilos should have approximately one liter of blood. Five minutes after the injection 125 c.c. of blood was withdrawn. The shed blood was kept for about five minutes and injected back into the animal's circulation. A sample of the shed blood was kept and found to be fluid 24 hours later. Again the animal showed no

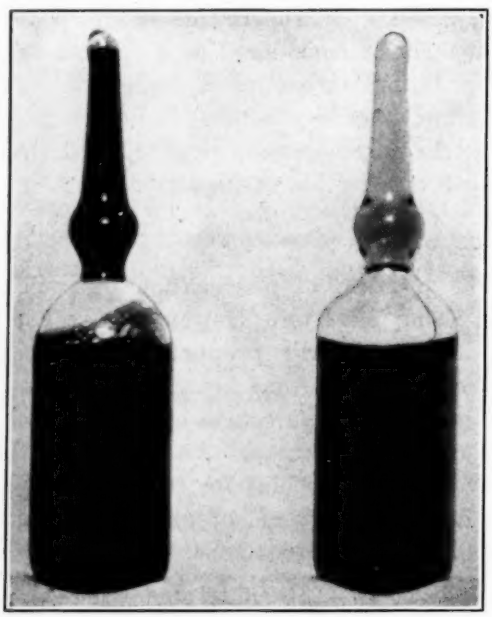

Fig. 1.

ill effects from the operation although the dose of heparin was 7.69 milligrams per pound of body weight.

The work was extended to observations on the human, and to date the anticoagulant has been used on 33 subjects in doses ranging from 50 to 300 milligrams. The dose most frequently used was 100 milligrams as that amount will prevent the coagulation of 500 c.c. of blood for 24 hours and therefore allows a safe margin for transfusions much above 500 c.c.

The use of heparin in the human gave results quite analogous to those observed in the animal studies except that often about 30 to 40 minutes after administration the patient developed symptoms which varied in intensity from mild headache and chilly sensation to intense headache, backache, vomiting, chills and temperature as high as 104.4 degrees. The cause of such untoward reactions is unknown. That it is due either to the anticoagulant or some substance associated with the anticoagulant is evidenced by the fact that the

reactions are experienced if the heparin be injected without the addition of blood, and, since different preparations vary greatly in their toxicity without markedly varying in their potency, it is suggested that the toxicity is due to an associated substance. Tests* have shown the material to be negative to the ninhydrin, biuret, Millons and acetic ferrocyanide tests for protein. The order of reaction is also against a protein reaction since patients often show a reaction with the first injection but not with subsequent injections. That the preservative is not the cause of the reaction is supported by observations with other preservatives (chloroform with evaporation at ice-box temperature) in which reactions were equally as common; also there are other preparations on the market in which saturated boric acid and .5 per cent chlorotone are used as preservatives, from which no reactions are experienced.

TABLE I

THE AMOUNT OF DELAY IN COAGULATION AND THE RETURN TO NORMAL

PATIENT	AMT. OF HEPARIN GIVEN	AMT. OF BLOOD GIVEN	AMT. OF BLOOD WITHDRAWN	COAGULATION BEFORE	COAGULATION AFTER	1 HOUR AFTER	1 HR. 30 MIN. AFTER	2 HOURS AFTER
No. 1	100 mg.	....	....	10 min.	33 min.	....	17 min.	....
No. 3	100 mg.	600 c.c.	....	8.5 min.	35 min.	....	....	Normal
No. 4	100 mg.	600 c.c.	....	7.0 min.	16 min.	....	7 min.	....
No. 12	100 mg.	500 c.c.	....	10.5 min.	....	14 min.	....	....
No. 18	200 mg.	600 c.c.	....	8 min.	....	12 min.	....	....
No. 19	200 mg.	....	150 c.c.	20 min.	60 min.	....	....	....
No. 26	100 mg.	....	350 c.c.	8 min.	41 min.	....	....	....
No. 33	75 mg.	....	....	11 min.	35 min.	22 min.	....	....

In the course of this study three different lots of ampules were used. The first gave reactions in 33⅓ per cent of the cases, the second gave 100 per cent reactions and the third gave 28 per cent reactions which for the most part were mild. The third lot of ampules represented a marked improvement in physical appearance as well as being the least toxic. Fig. 1 is presented to show the marked decrease in color as well as an absence of surface foam. Such a product has resulted through a slight modification in the mode of preparation.

The Delay Produced in Coagulation and the Time Necessary for Coagulation to Return to Normal.—In the use of such a potent anticoagulant for blood transfusions it is important that one know its effect on the coagulation of the recipient's blood. Therefore observations were made in which the recipient's coagulation time was determined before transfusion, immediately after transfusion and at varying periods following transfusion. Table I

*Made in the laboratory of Hynson, Westcott and Dunning.

shows briefly (1) the amount of heparin given, (2) the coagulation time before administration and after administration; (3) also the amount of blood given with the heparin or the amount of blood withdrawn from the subject, whichever the procedure may have been. The results rather closely agree with those obtained from animal observations in that the return to normal coagulation requires about 1 hour and 45 minutes to 2 hours.

Although our experience is quite limited there appears, from the cases studied, no marked increase in risk from hemorrhage following doses up to 100 milligrams. In one case markedly jaundiced there was actually an improvement in coagulation. Before receiving 500 c.c. of blood containing 100 milligrams of heparin the patient's blood showed a poorly formed clot in 20 minutes, and 30 minutes following the transfusion a good clot formed in 18 minutes.

CONCLUSIONS

1. Heparin as used in the early part of the work proved too toxic to warrant its routine clinical use in doses of 100 milligrams or above.

2. Subsequent preparations have given encouraging results, and it appears that with more work on the subject heparin may prove a desirable anticoagulant for clinical use.

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THE USE OF DEFIBRINATED BLOOD PRESERVED WITH FORMALDEHYDE FOR THE PURPOSE OF CONTROLLING DILUTIONS OF DRIED BLOOD*

By Ruth Gilbert, M.D., and Ellsworth A. Parsons, Albany, N. Y.

FOR several years, the problem of providing a satisfactory standard with which to compare dilutions of dried blood submitted for agglutination tests has presented considerable difficulty. Although, in this laboratory, the number of specimens received in the tube outfits is increasing, the majority of specimens are still submitted on slides. It is impracticable to estimate the amount of blood contained in such specimens, and therefore the color of dilutions made from them must be compared with that of dilutions which are known to be exact.

Only one method of diluting dried blood accurately has been found in a review of the literature. This is the method of Ruediger and Hulbert[1]

*From the Division of Laboratories and Research, New York State Department of Health, Albany. Augustus Wadsworth, M.D., Director.
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who suggest air-drying the blood for twenty-four hours and then weighing, using 0.1 c.c. of salt solution per milligram of blood for a 1:25 dilution. The impracticability of this method for routine work is obvious.

Heretofore, in this laboratory, known dilutions of dried human blood have been used in preparing color standards. Dried blood, however, usually assumes a brownish tint after forty-eight hours. Also, at times, parts of the specimen may peel off the slides.

The preparation of accurate dilutions of fresh blood each day is inconvenient, and it was therefore, considered advisable to investigate the use of defibrinated blood. Accordingly, a small amount of sheep's blood was defibrinated by shaking it in a bottle containing sterile glass beads for five or ten minutes, and to 3 c.c. of this, 0.15 c.c. of a 1:20 dilution of formalin (40 per cent formaldehyde) was added. The first specimen prepared remained satisfactory for a period of four weeks.

For purposes of confirmation and comparison, twelve different specimens of defibrinated sheep's blood were obtained on different days and treated with formalin. They were then diluted every other day for a period of two weeks. During that time, all were found to compare satisfactorily with accurate dilutions of freshly drawn human blood.

The dilutions are prepared in the concavities of a porcelain plate of the type used for diluting the specimens. Forty-five hundredths of a cubic centimeter of 0.5 per cent salt solution are pipetted into the first concavity, 0.03 c.c. into the second, and 0.09 c.c. into the third. Then, by adding 0.05 c.c. of the defibrinated sheep's blood to the first concavity, a dilution of 1:10 is obtained. Three hundredths of a cubic centimeter of the 1:10 dilution added to the second and third concavities gives a 1:20 and a 1:40 dilution respectively.

The specimen of dried blood is emulsified on the slide in a small amount of salt solution and transferred to one of the first concavities in a porcelain plate. More salt solution is then added if necessary until the color approximates as nearly as possible that of the 1:10 dilution of the defibrinated sheep's blood. The 1:20 and 1:40 dilutions are then prepared in the second and third concavities and the specimen is ready to be tested.

This method has been employed in the routine work and has proved to be a simple procedure for preparing color standards for use when specimens of dried blood are diluted for the agglutination test.

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HISTOPATHOLOGIC AND BLOOD STUDIES WITH SILVER-SALVARSAN*

By C. W. Hooper, M.D., and C. N. Myers, Ph.D., New York City

THIS is the third of a series of articles dealing with the retention and elimination of silver after the administration of silver compounds. In the first article of the series, Myers, 1923, carefully reviewed the literature, presented the reported cases of argyria in chronologic order and discussed the nature of the silver products that had been found to be the causative agents. The second article, by Myers and Corbitt, 1924, was an experimental study based upon the results obtained with 21 rabbits and approximately 220 rats. They compared the retention and elimination of the silver contained in silver-salvarsan with that contained in argyrol and cargentos. They found that after single massive intravenous injections of 200 milligrams silver-salvarsan per kilogram (equivalent to a single clinical dose of 12 grams) the albino rat excretes 96.5 per cent of the silver injected within six days. In comparable experiments in which they employed argyrol and cargentos it was found that about 20 times as much silver is retained on the sixth day in the case of argyrol, and 15 times in the case of cargentos as in silver-salvarsan. They also injected 21 rabbits with repeated doses of silver-salvarsan. The 17 rabbits that received 66.7 milligrams per kilogram repeatedly, excreted on the average over 90 per cent of the total amount of silver injected within a period of seven days after the last injection. The four rabbits that received 10 milligrams per kilogram excreted approximately all of the silver injected within the same period.

The purpose of this article is to describe the histopathologic changes found at necropsy in 20 of the rabbits and to present the blood counts and hemoglobin figures taken at weekly intervals during the entire course of the injections in eight of the rabbits.

EXPERIMENTAL

Twenty rabbits were employed as experimental animals. The silver-salvarsan was administered by injection from a syringe into one of the ear veins. One or two per cent solutions of the drug in freshly distilled water were used for the injections. In a few of the experiments 0.4 per cent sodium chloride was added. Sixteen of the rabbits received a dose of 66.7 milligrams per kilogram repeated at intervals of three to seven days. Four of the rabbits received 10 milligrams per kilogram repeated at the same intervals. The minimal amount of the drug injected was 223 milligrams in a period of 47 days and the maximal amount 2,363 milligrams in 70 days. The silver-

*From the Department of Experimental Medicine of the H. A. Metz Laboratories. Received for publication, June 6, 1924.

salvarsans employed, controls SS9 and SSH, were commercial preparations which had passed the official standard for toxicity. The observed arsenic contents were 21.0 and 19.31 per cent, respectively, and the silver contents were 14.5 and 14.3 per cent, respectively. After an interval of 6 to 44 days after the last injection the animals were sacrificed with chloroform and immediately necropsied. Three of the animals died within four days after the last injection of the drug from acute bronchopneumonia. Small pieces of the following organs were saved for section: cerebrum, bone marrow, lungs, heart, stomach, small intestine, spleen, adrenals and kidneys.

HISTOLOGIC EXAMINATION OF TISSUES

For histologic detail, the tissues were fixed in Zenker-formalin fluid, washed thoroughly in running water, hardened in alcohol, cut in paraffin, and sections stained with hematoxylin and eosin. The term Zenker-formalin fluid is used to indicate Zenker's fluid without the acetic acid, to which 10 per cent of neutral 4 per cent formaldehyde solution is added immediately before use.

For the microchemical demonstration and differentiation of silver, sections were first submerged in a saturated solution of iodine in alcohol for five minutes to dissolve any mercuric oxide crystals. They were then treated with a warmed aqueous solution of hydrogen sulphide for ten minutes to darken the silver granules and finally stained with hematoxylin and eosin. Other sections were treated with a strong aqueous solution of potassium cyanide for ten minutes to dissolve the silver granules.

Perl's reaction was employed to eliminate the possibility of iron. The sections were subjected for 10 minutes to a mixture of a 2 per cent solution of potassium ferrocyanide, one part, and a 1 per cent solution of hydrochloric acid, three parts, heated to 60° C., after which the sections were differentiated in 0.5 per cent hydrochloric acid solution and stained with neutral red and eosin.

BLOOD EXAMINATIONS

The rabbits were fasted sixteen to twenty hours immediately before the blood examinations. The blood was obtained from a freely bleeding ear puncture. The silver-salvarsan was administered uniformly after the blood had been obtained for the counts.

The hemoglobin determinations were made by means of the Sahli hemoglobinometer. The color tube of the hemoglobinometer was standardized against blood having an oxygen capacity of 18.5 volumes per cent by the Van Slyke gasometric method, 1918 (approximately 14 grams hemoglobin per 100 c.c.).

The red and white cell counts and differential white cell counts were made in the routine manner. The number of nucleated red corpuscles seen in differentially counting 200 white cells was recorded.

Table I presents a summary of the individual protocols of rabbits 2 to 13 inclusive. The total amount of silver-salvarsan injected varied from 469 to 2,300 milligrams containing a total amount of silver varying from 67.5 to 331.2 milligrams. The total amount of silver retained (found by Myers after

TABLE I

SILVER-SALVARSAN INTRAVENOUSLY—REPEATED ADMINISTRATION—66.7 MILLIGRAMS PER KILOGRAM

RABBITS 2-13

NO.	NUMBER OF INJECTIONS	TOTAL AMOUNT SILVER-SALVARSAN INJECTED	TOTAL AMOUNT SILVER INJECTED	TOTAL SILVER RETAINED		DURATION OF EXPERIMENT	INTERVAL AFTER LAST INJECTION	WEIGHT			RESULT
				AMOUNT	PER CENT			INITIAL	AT LAST INJECTION	FINAL	
		mg.	mg.	mg.		days	days	grams	grams	grams	
2	7	1030	149.3	38.8	25.98	44	8	2455	1765	1310	Killed
3	13	1554	225.33	21.6	9.59	52	10	2410	1670	1735	Killed
4	13	1236	179.2	8.4	4.70	62	7	2520	2290	2240	Killed
5	15	1594	231.13	7.5	3.26	55	7	2270	1755	1795	Killed
6	19	1661	240.85	19.1	7.93	78	7	1575	1600	1650	Killed
7	13	1520	225.4	24.1	10.70	51	7	1720	1940	2020	Killed
8	19	2300	331 2	43.5	13.10	76	12	1935	1885	1995	Killed
9	9	1169	168.3	5.9	3.50	36	6	1815	2030	1980	Killed
10	10	1415	203.8	43.4	21.30	37	5	2170	1900	1540	Died
11	5	469	67.54	12.3	18.20	19	4	1410	1460	1180	Killed
12	9	1217	175.3	14.8	8.44	32	3	2130	1800	1657	Died
13	11	1249	179.86	8.8	4.90	53	16	1755	1800	2120	Killed

incineration) varied from 5.9 to 43.5 milligrams and represented from 3.5 to 25.98 per cent of the total amount administered. Five of the animals gained in weight during the course of the injections and seven lost in weight. Two of the animals died of an intercurrent bronchopneumonia before the completion of the experiment. Nine were sacrificed with chloroform at the end of the experiment.

Rabbit 2 (March 19, 1921). Necropsy.—Animal is poorly nourished. Skin, mucous membranes and conjunctivae are intact and show no abnormal pigmentation. Subcu-

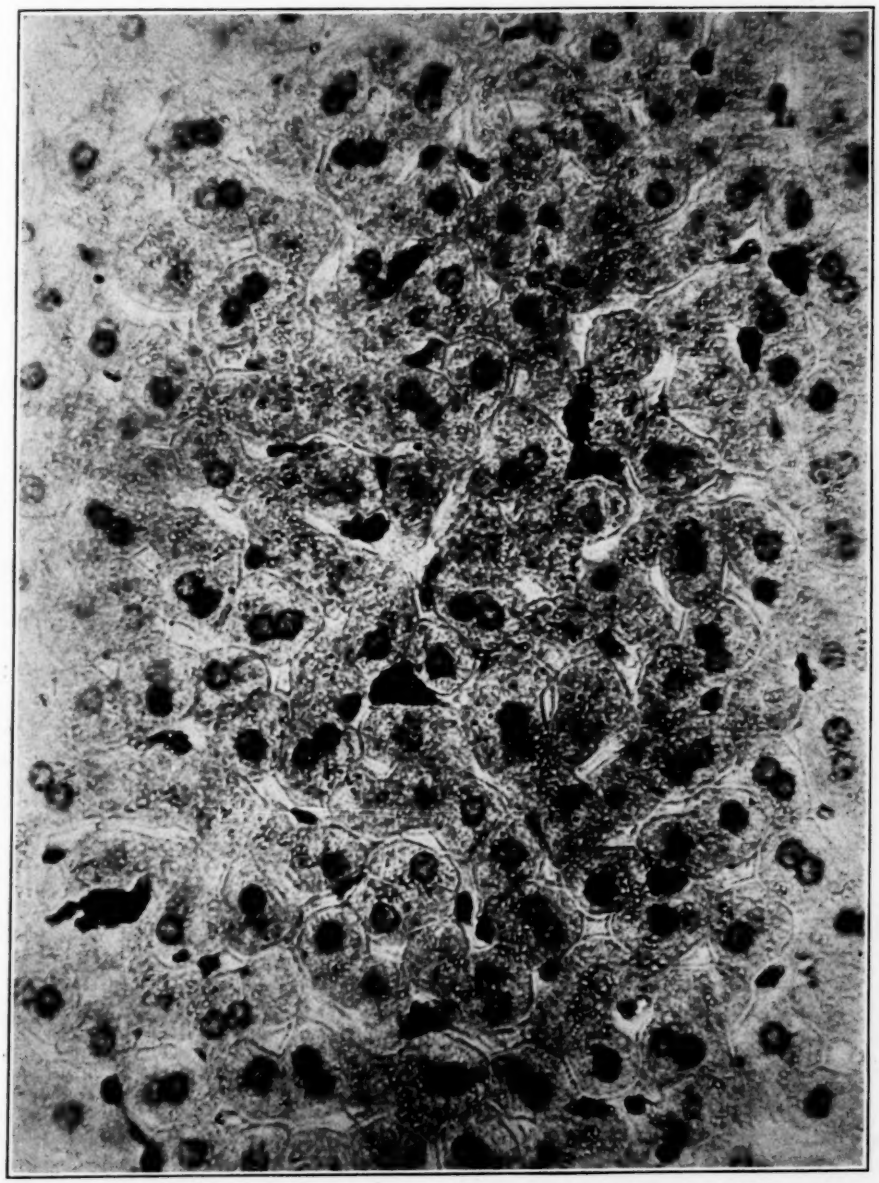

Fig. 1.—Liver from rabbit given nineteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram (20 times the average clinical dose) within 64 days. Silver granules are deposited in the Kupffer cells and in extracellular groups between the liver cell columns. They appear black in microphotograph. High dry lens magnification.

taneous and omental fats are scanty though normal in color. Serous surfaces are normal. No increase in serous fluids. Blood clots normally. *Heart* normal in gross. Microscopically the endomysium contains a few isolated silver-holding phagocytes. *Lungs* contain many small areas of bronchopneumonia. No abnormal pigmentation in gross or in section. *Stomach* and *intestines* appear normal in gross. Microscopically the tunica propria (stomach and small intestine) contains many groups of silver granules and a few silver-holding phagocytes. The silver granules are situated almost entirely beneath the lining epithelium. *Liver* shows a definite increased pigmentation. The cut section is deep chocolate brown in color. Gall bladder and bile ducts are normal. Microscopically the liver cells around the efferent veins contain fat droplets, the periportal connective tissue is increased, the bile ducts are proliferated and there is an accumulation of small round cells. The Kupffer cells are richly loaded with dark brown silver granules. There are also many extracellular groups of silver granules between the liver cell columns and in the periportal connective tissue. A few of the parenchyma cells contain silver granules. *Spleen* appears normal in size. On cut section it is chocolate brown in color. Microscopically the pulp is rarefied and contains many free groups of silver granules and a few silver-holding phagocytes. Malpighian bodies are normal. *Pancreas* normal in gross. *Adrenals* normal in gross and in section. *Kidneys* are somewhat swollen. On cut section the capsule bulges, striations are regular although somewhat obscured. Microscopically the parenchyma cells are swollen and granular. Glomeruli appear normal. Interstitial tissue contains many fine silver granules. *Bone marrow* of femur is granular and uniformly reddish chocolate brown in color. Microscopically it is cellular, vascular and contains only a few fat cells. There are numerous phagocytes heavily loaded with silver granules and groups of silver granules lying free in the tissue. Brain and meninges appear normal in gross. Cerebrum normal in section.

Rabbit 3 (May 4, 1921). Necropsy.—Animal is poorly nourished. Skin, mucous membranes, conjunctivae and serous surfaces show no abnormal pigmentation. Heart and lungs are normal in gross and in section. There is an excess of peritoneal fluid. *Liver* is definitely cirrhotic and deep chocolate brown in color. Gall bladder and bile ducts appear normal. Microscopically the Kupffer cells contain an abundance of silver granules. There are also extracellular groups of silver granules between the liver cell columns and in the periportal connective tissue. *Spleen* shows increased pigmentation. The pulp contains many free groups of silver granules and a few silver-holding phagocytes. *Stomach* and *small intestine* normal except for the silver granules in the tunica propria. The groups of silver granules and silver-containing phagocytes are situated beneath the lining epithelium. *Pancreas* and *adrenals* are normal. *Kidneys* normal except for increased pigmentation. The silver granules are situated in the interstitial tissue. *Bone marrow* of femur is uniformly deep chocolate reddish brown in color. Microscopically it is very cellular and heavily sprinkled with silver phagocytes and extracellular groups of silver granules. Brain and meninges normal.

Rabbit 4 (May 26, 1921). Necropsy.—Animal is fairly well nourished. Serous cavities are normal. Heart and lungs normal in gross and in sections. Stomach and small intestine are normal. *Liver* and *spleen* normal except for slight increased pigmentation. Microscopically some of the Kupffer cells contain silver granules. The spleen pulp contains many free groups of silver granules and a few silver-holding phagocytes. Gall bladder and bile ducts normal. Pancreas and adrenals normal. Kidneys are normal except for slight increased pigmentation. The silver granules are contained in the interstitial tissue of both the cortex and medulla. *Bone marrow* of femur is deep chocolate reddish brown in color. Microscopically it contains many groups of silver granules.

Rabbit 5 (May 19, 1921). Necropsy.—Liver, spleen and kidneys show increased pigmentation. Kupffer cells, spleen pulp and interstitial tissue of kidneys contain silver granules. The kidneys contain a few scattered small areas radially arranged, which show marked interstitial lesions, accompanied by epithelial changes and accumulation of round cells. Bone marrow of femur uniformly deep chocolate brown. Microscopically the marrow is heavily sprinkled with groups of silver granules.

Rabbit 6 (June 13, 1921). Necropsy.—Skin, mucous membranes, conjunctivae show no abnormal pigmentation. Serous cavities normal. Heart and lungs normal in gross and in sections. Liver and kidneys show increased pigmentation. Gall bladder and bile ducts normal. Stomach and small intestine contain groups of silver granules in the tunica propria. Bone marrow of femur is hyperplastic and shows the usual silver granules. Necropsy is otherwise negative.

Rabbit 7 (May 27, 1921). Necropsy.—Negative except for increased pigmentation of the liver, kidneys, spleen and bone marrow accompanied microscopically with the deposition of silver granules. The Malpighian bodies in the spleen as well as the stroma con-

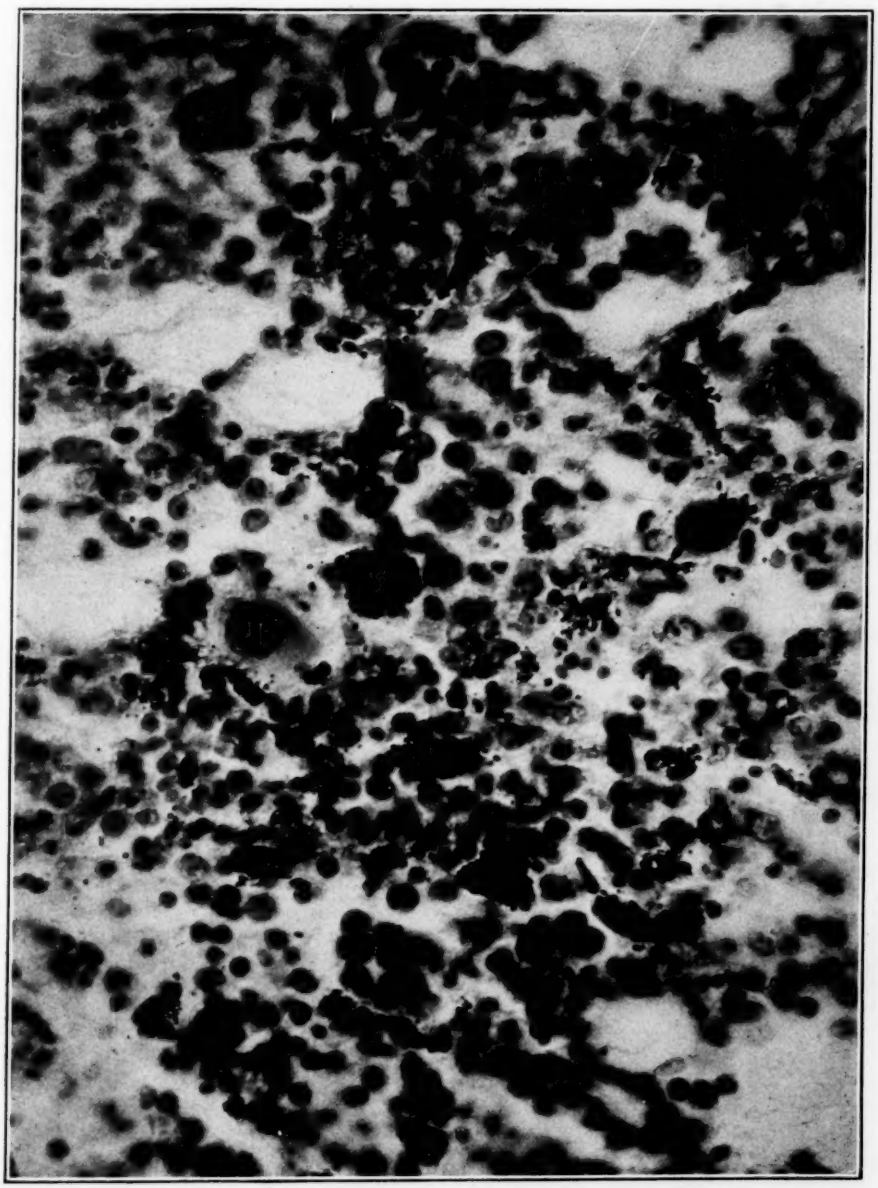

Fig. 2.—Bone marrow from rabbit given thirteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 42 days. Silver granules are deposited in extracellular groups and in many phagocytes. High dry lens magnification.

tain many groups of silver granules. The kidneys show a few radially arranged areas of cellular infiltration. The intervening glomeruli and parenchyma cells are normal.

Rabbit 8 (July 5, 1921). Necropsy.—Skin, mucous membranes and conjunctivae normal. Blood flows and clots normally. Serous cavities normal. Heart and lungs normal and show no silver microscopically. Liver, spleen and bone marrow are heavily pigmented. Microscopically the silver granules show the usual distribution. The tunica propria of the stomach and small intestine contains many groups of silver granules. Adrenals are normal in gross and in section. Brain and meninges are normal.

Rabbit 9 (June 6, 1921). Necropsy.—Negative except for the increased pigmentation

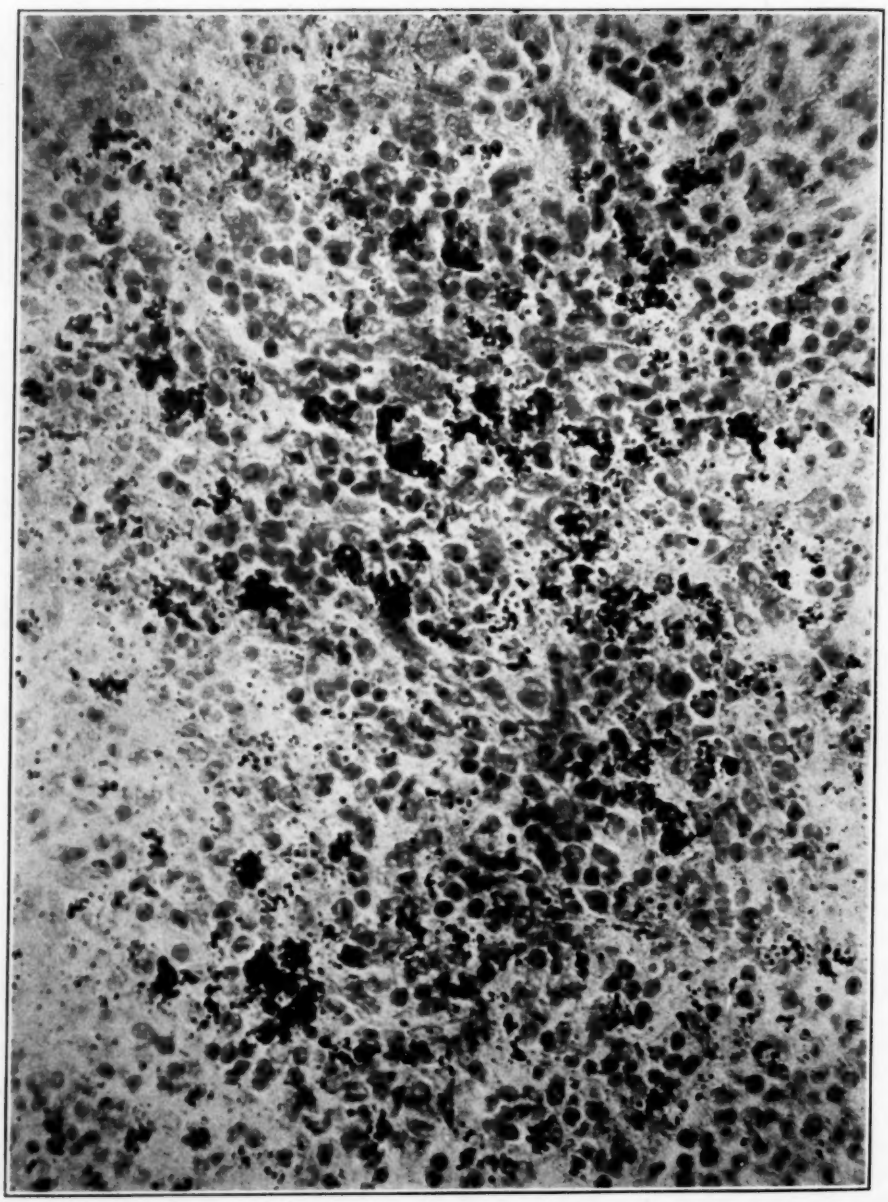

Fig. 3.—Spleen from rabbit given ten intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 32 days. The pulp contains many extracellular groups of silver granules and a few silver-holding phagocytes. High dry lens magnification.

of the liver, spleen, kidneys and bone marrow. Microscopically the deposition of silver granules is not as extensive as in Rabbit 8.

Rabbit 10 (June 14, 1921). Necropsy.—Animal is poorly nourished. Considerable postmortem degeneration. Some excess of pleural fluid. *Lungs* show extensive bronchopneumonia. The upper lobe of the left lung is completely consolidated. Heart is normal. Stomach and intestines appear normal. Microscopically the tunica propria of the stomach and small intestine contains many groups of silver granules and a few silver-holding phagocytes. *Spleen* on section is chocolate brown in color. Microscopically the pulp contains many extracellular groups of silver granules and a few silver-holding phagocytes.

Fig. 4.—Kidney from a rabbit given thirteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 42 days. The silver granules are deposited in the interstitial tissue. High dry lens magnification.

Liver shows a markedly increased pigmentation. Gall bladder and bile ducts normal. Liver sections show a definite increase in periportal connective tissue with an accumulation of round cells. Some of the liver lobules are completely surrounded by a narrow band of fibrous tissue. The Kupffer cells are loaded with silver granules and there are many extracellular groups of silver granules between the liver cell columns and the interlobular connective tissue. *Kidneys* are swollen and show an increased pigmentation. The kidney sections show silver granules in the interstitial tissue. The tubular epithelium is swollen and granular and shows no necrosis. Some of the capsular spaces of the glomeruli

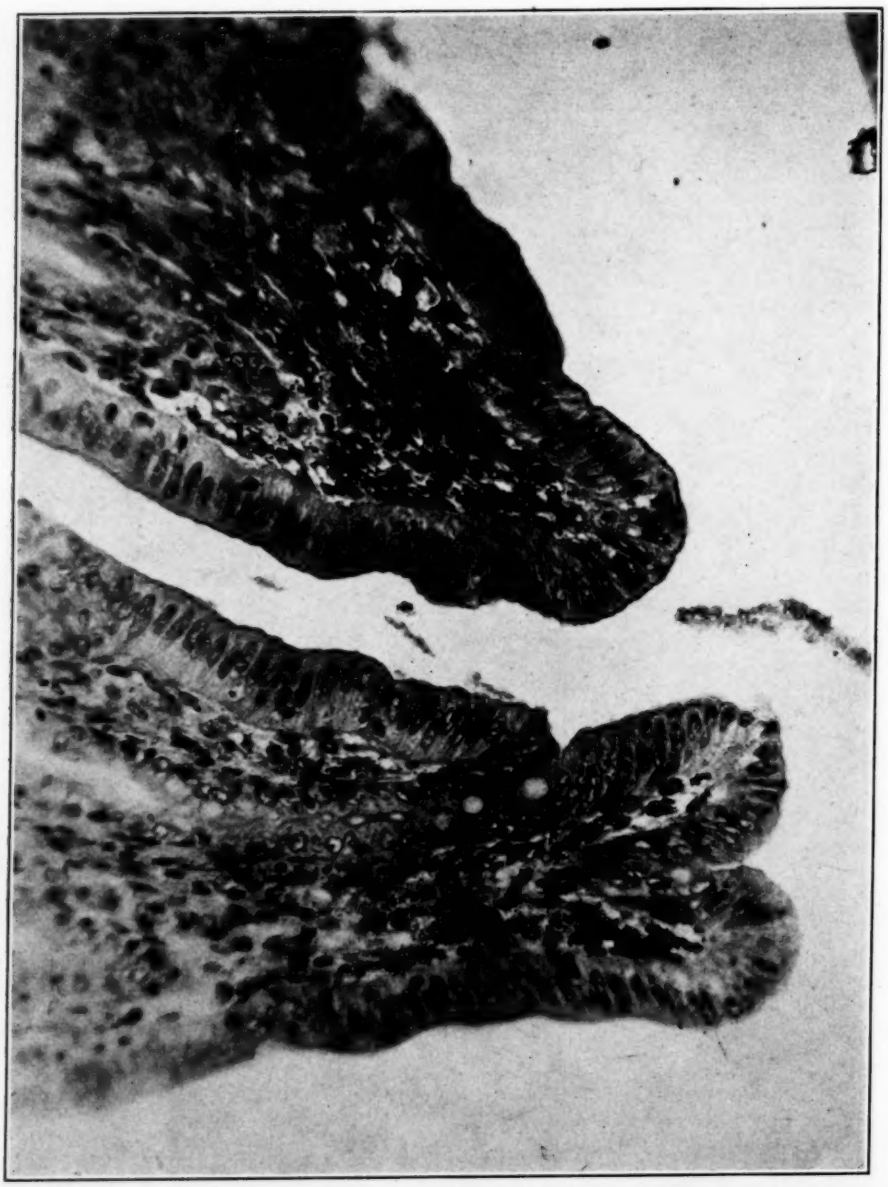

Fig. 5.—Villi of small intestine from a rabbit given fifteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within 45 days. The corium contains extracellular groups of silver granules and few silver-holding phagocytes. High dry lens magnification.

contain an albuminous precipitate. *Bone marrow* of femur is reddish chocolate brown throughout. Microscopically it is very cellular and contains a large amount of silver.

Rabbit 11 (May 27, 1921). Necropsy.—Animal is poorly nourished. No abnormal pigmentation of the skin or mucous membranes. Heart is normal. *Lungs* show a few small areas of bronchopneumonia. Stomach, intestines and adrenals are normal. *Liver* is cirrhotic. Gall bladder and bile ducts normal. Microscopically many of the Kupffer cells contain silver granules and also a few of the parenchyma cells. There are also a few granules in the increased periportal connective tissue. *Spleen* is normal in size and shows only a slight increased pigmentation. Microscopically the pulp contains a few free groups of silver granules and also a few pigment-holding phagocytes. *Kidneys* are somewhat swollen and show a slight increased pigmentation. Microscopically the parenchyma cells are swollen and granular. The glomeruli appear normal. There are a few silver granules in the basement membrane of the tubules and interstitial connective tissue. Bone marrow of femur is hyperplastic and shows considerable silver. Brain and meninges are normal. Cerebrum sections normal.

Rabbit 12 (June 19, 1921). Necropsy.—Animal is poorly nourished. Heart is normal, the right auricle is filled with firm blood clot. *Lungs* show extensive bronchopneumonia. Thymus is normal in gross and in sections. Liver is cirrhotic. Liver sections show the usual silver deposit. Spleen sections are normal except for the groups of silver granules scattered through the stroma. Adrenals are normal. *Kidneys* are somewhat swollen and show a slight increased pigmentation. Microscopically the kidney cells are swollen and granular and show no necrosis. The basement membrane of the tubules and interstitial tissue contains silver granules. Bone marrow is quite cellular and heavily sprinkled with small groups of silver granules. Brain and meninges are normal.

Rabbit 13 (July 27, 1921). Necropsy.—Negative except for the increased pigmentation of the liver, spleen, bone marrow and kidneys. Kidney sections show several areas of cellular infiltration. Bone marrow of femur is uniformly hyperplastic and heavily pigmented.

Rabbit 14. Necropsy.—Skin, mucous membranes and conjunctivae normal. Serous surfaces normal. Heart is normal. Heart sections show no silver. Lungs are negative except for a few silver granules in the interlobular connective tissue. *Stomach* and *intestines* are normal in the gross. Microscopically there are a few groups of silver granules in the tunica propria of the stomach and small intestine. *Spleen* sections show a few groups of silver granules in the pulp. Liver shows an increase in periportal connective tissue and many of the Kupffer cells contain silver granules. Kidneys are normal. Microscopically the connective tissue stroma contains a few silver granules. Bone marrow of femur is cellular and contains several groups of silver granules. Brain and meninges are normal.

Rabbit 15. Necropsy.—Negative except for slight increased pigmentation of the liver, spleen, kidneys and bone marrow.

Rabbit 16. Necropsy.—Skin, mucous membranes, conjunctivae and serous surfaces normal. Thymus, heart and lungs normal. Stomach and duodenum sections show a few groups of silver granules in the fibrorecticular stroma of the corium. *Liver* is cirrhotic. Kupffer cells and periportal connective tissue contain silver granules. Spleen pulp contains a few groups of silver granules. Adrenals are normal. *Kidneys* show scattered, radially arranged interstitial lesions with the formation of small depressions on the outer surface. These areas also show epithelial alterations, with infiltration of round cells. In the non-affected areas the parenchyma cells and glomeruli are normal. Bone marrow of femur appears somewhat hyperplastic and contains many groups of silver granules. Brain and meninges normal. Cerebrum sections show no change.

Rabbit 17. Necropsy.—No external abnormal pigmentation. Serous cavities are normal. Heart and lungs normal. Stomach, small intestines, liver, spleen and kidneys contain deposits of silver granules. Bone marrow is of the mixed type and contains many groups of silver granules. Necropsy otherwise negative.

TABLE II

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 2.534 GRAMS. TOTAL AMOUNT OF SILVER GIVEN = 362.4 MG. PER CENT OF SILVER RETAINED = 2 (MYERS)

RABBIT 14

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	115	1730	70	5,080,000	0.70	7,000	37.5	5.5	55.5	1.5	0
4	113	1690									
7	114	1705	65	4,408,000	0.74	12,000	57.0	6.0	34.5	2.5	0
10	120	1795									
14	125	1850	67	4,544,000	0.74	11,800	55.0	6.0	35.0	4.0	1
17	126	1840									
21	127	1900	80	4,848,000	0.83	11,950	48.5	7.5	38.5	5.5	0
24	127	1910									
28	124	1860	85	5,928,000	0.72	14,950	59.0	5.5	30.0	5.5	0
31	127	1905									
35	134	2015	94	5,664,000	0.84	9,600	39.0	8.0	48.5	4.5	0
38	134	2010									
42	141	2120	103	6,520,000	0.79	12,250	47.0	6.5	37.0	9.5	0
45	142	2125									
49	149	2230	98	6,224,000	0.79	10,900	42.0	6.0	49.5	2.5	0
52	149	2230									
56	152	2275	97	6,848,000	0.71	9,200	43.0	4.5	46.5	6.0	0
59	153	2290									
63	162	2430	87	5,464,000	0.81	7,350	46.5	5.0	45.5	3.0	1
69		2400	84	5,568,000	0.76	9,900	62.0	7.5	29.5	1.0	2
77		2410	89	5,528,000	0.81	8,450	51.0	5.5	38.0	5.5	0
83		2400	91	5,936,000	0.77	8,650	48.5	6.0	41.5	4.0	0
91		2475	95	5,720,000	0.83	14,150	51.5	5.5	40.5	2.5	0
98		2455	88	5,328,000	0.83	9,650	55.5	7.5	30.5	6.5	0
101		2380	92	5,488,000	0.85	10,800	43.5	4.5	48.5	3.5	0
105		2440	88	5,344,000	0.83	11,600	54.5	3.0	40.0	2.5	0

105th day (October 25, 1921) Active and apparently normal. Sacrificed with chloroform.

TABLE III

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 2.369 GRAMS. TOTAL AMOUNT OF SILVER GIVEN = 238.8 MG. PER CENT OF SILVER RETAINED = 1.52 (MYERS)

RABBIT 15.

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	127	1905	92	6,572,000	0.71	13,900	39.5	6.5	50.5	3.5	0
4	135	2030									
7	137	2060	97	6,904,000	0.70	12,450	45.5	5.0	48.0	1.5	0
10	136	2040									
14	138	2070	96	6,760,000	0.72	10,200	59.0	13.5	23.5	4.0	0
17	138	2070									
21	144	2140	94	7,056,000	0.67	9,450	46.5	7.5	43.5	2.5	0
24	154	2315									
28	149	2235	96	7,360,000	0.66	7,800	56.5	4.5	32.5	6.5	0
31	149	2240									
35	146	2200	96	6,936,000	0.70	12,050	37.0	8.0	46.0	9.0	0
38	163	2440									
42	162	2410	97	6,752,000	0.72	7,800	56.5	4.0	36.5	3.0	0
45	160	2400									
49	165	2480	90	5,800,000	0.78	6,800	57.5	1.5	34.0	7.0	1
52	166	2490									
56		2525	85	5,336,000	0.80	10,000	54.5	7.0	34.0	4.5	0
63		2635	88	5,480,000	0.81	9,100	50.5	7.0	31.5	11.0	0
69		2640	84	5,072,000	0.84	11,150	53.5	5.5	35.0	6.0	0
77		2785	89	5,600,000	0.80	8,900	69.0	5.5	22.5	3.0	0
83		2790	95	5,504,000	0.86	9,000	58.0	6.5	34.5	1.0	0
91		2890	100	6,360,000	0.79	11,500	68.0	6.0	22.0	4.0	0

91st day (October 14, 1921) Active and apparently normal. Killed with chloroform.

TABLE IV

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 2.363 GRAMS. TOTAL AMOUNT SILVER GIVEN = 337.9 MG. PER CENT OF SILVER RETAINED = 1.2 (MYERS)

RABBIT 16

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	116	1745	86	7,592,000	0.57	14,300	53.0	8.0	37.0	2.0	0
4	119	1765									
7	118	1765	86	7,248,000	0.60	10,110	40.5	6.5	50.5	2.5	0
10	114	1715									
14	118	1770	79	6,528,000	0.61	10,800	45.5	6.5	46.0	2.0	0
17	117	1750									
21	116	1740	80	6,304,000	0.63	15,200	43.5	8.0	43.0	5.5	0
24	117	1750									
28	116	1745	79	6,048,000	0.65	22,000	51.0	5.0	40.5	3.5	2
31	116	1750									
35	115	1720	83	6,032,000	0.69	12,400	46.0	8.0	39.0	7.0	4
38	117	1755									
42	119	1770	88	7,000,000	0.63	13,600	44.5	4.0	48.5	3.0	1
45	120	1795									
49	120	1790	83	6,520,000	0.64	18,850	39.0	4.0	55.5	1.5	4
52	123	1790									
56	117	1750	81	6,136,000	0.66	16,650	49.0	2.5	45.5	3.0	0
59	120	1800									
63	122	1835	84	6,856,000	0.62	15,900	45.0	12.5	40.0	2.5	0
66	123	1845									
70		1875	80	6,512,000	0.62	14,950	36.0	8.0	53.5	2.5	1
78		1866	95	7,212,000	0.65	12,100	40.0	6.0	50.0	4.0	0
84			100	7,808,000	0.64	11,100	38.0	6.5	47.5	8.0	0
91		1866	103	8,000,000	0.64	15,300	44.5	5.0	47.5	3.0	0
101		1870	94	7,128,000	0.66	12,200	45 5	6.5	44.5	3.5	0
105		1920	91	6,896,000	0.67	11,600	55.5	3.5	39.0	2.0	0
108		1950	95	6,840,000	0.69	13,000	43.0	2.5	53.0	1.5	0

108th day (October 28, 1921). Good condition and active. Sacrificed with chloroform.

TABLE V

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 1.12 GRAMS. TOTAL AMOUNT OF SILVER GIVEN = 160.2 MG. PER CENT SILVER RETAINED = 2.5 (MYERS)

RABBIT 17

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	113	1700	84	7,000,000	0.60	8,100	43.0	8.5	45.5	3.0	0
4	115	1730									
7	120	1795	88	7,240,000	0.61	8,900	48.0	5.0	45.0	2.0	0
10	120	1800									
14	121	1820	81	6,068,000	0.68	7,250	45.5	16.5	33.5	4.5	0
17	117	1730									
21	108	1620	76	5,604,000	0.68	12,100	50.5	6.5	41.0	2.0	2
24	109	1630									
28	100	1495	76	6,096,000	0.63	9,950	37.0	5.5	55.5	2.0	0
31	97	1455									
35		1340	80	6,112,000	0.66	10,200	46.5	6.5	45.0	2.0	0
42		1270	86	7,128 000	0.61	12,000	32.5	1.5	64.0	2.0	0
49		1490	75	6,304.000	0.60	9,300	59.5	2.0	32.5	6.0	0
56		1630	79	6,272 000	0.64	10,850	50.0	4.5	43.5	2.0	0
63		1740	87	7,072,000	0.62	15,800	58.0	3.5	35.5	3.0	0
69		1860	83	6,456 000	0.65	9,600	57.0	4.0	36.5	2.5	0
73		1870	80	6,080,000	0.67	6,500	43.5	3.0	52.0	1.5	0

73rd day (September 23, 1921) Active. Killed with chloroform.

TABLE VI

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT SOLUTION IN 0.4 PER CENT SODIUM CHLORIDE. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.276 GRAMS. TOTAL AMOUNT SILVER GIVEN = 39.5 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS).

RABBIT 18

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	21.0	2100	96	7,608,000	0.63	9,150	61.5	3.5	31.5	3.5	0
5	20.8	2080									
8	21.8	2180	100	7,784,000	0.65	8,600	69.0	4.0	24.5	2.5	0
12	21.6	2170									
15	21.6	2165	101	7,088,000	0.72	8,550	67.5	4.0	27.0	1.5	0
19	22.8	2280									
22	22.8	2290	98	6,456,000	0.77	9,450	63.0	2.5	30.0	4.5	0
26	23.6	2365									
29	24.0	2410	99	6,064,000	0.83	7,650	71.5	2.5	24.0	2.0	0
33	25.0	2465									
36	25.0	2485	102	6,584,000	0.78	9,550	64.5	2.0	30.0	3.5	0
40	26.0	2640									
43		2660	99	6,144,000	0.81	7,200	62.0	1.5	32.0	4.5	0
50		2740	104	6,672,000	0.79	11,050	68.0	4.5	22.5	5.0	0
57		2830	107	7,952,000	0.68	13,350	55.5	3.0	34.5	7.0	0
64		2890	105	7,672,000	0.69	13,450	69.5	3.0	25.0	2.5	0
71		2940	103	6,744,000	0.77	9,450	62.5	3.5	29.0	5.0	0
78		2960	104	6,448,000	0.81	12,200	64.5	7.0	24.0	4.5	0
82		2930	104	6,208,000	0.84	10,550	47.0	2.5	45.0	5.5	0
84		3010	102	6,552,000	0.78	7,650	63.5	2.0	31.5	3.0	0

84th day (November 3, 1921) Excellent condition. Sacrificed with chloroform.

TABLE VII

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.223 GRAM. TOTAL AMOUNT OF SILVER GIVEN = 31.9 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBIT 19

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	16.4	1635	85	5,944,000	0.72	9,050	53.5	5.0	37.5	4.0	0
5	17.4	1735									
8	17.0	1695	85	5,968,000	0.72	10,000	67.0	2.5	26.5	4.0	0
12	18.8	1820									
15	18.0	1800	93	6,200,000	0.75	14,050	60.5	2.5	34.0	3.0	0
19	18.4	1840									
22	18.4	1830	85	5,552,000	0.77	11,100	62.0	1.5	33.0	3.5	0
24	19.0	1900									
29	19.0	1895	88	6,072,000	0.73	15,050	56.0	4.5	35.5	4.0	0
33	20.0	1995									
36	19.6	1965	96	6,248,000	0.77	13,350	60.0	10.0	24.5	5.5	0
40	21.0	2090									
43		2115	95	6,440,000	0.74	10,850	61.5	2.0	33.5	3.0	0
47		2200	98	6,216,000	0.79	11,900	67.0	5.0	23.0	5.0	0

47th day (September 27, 1921). Active and normal. Killed with chloroform.

TABLE VIII

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT SOLUTION IN 0.4 PER CENT SODIUM CHLORIDE. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.297 GRAMS. TOTAL AMOUNT SILVER GIVEN = 42.5 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBITT 20

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	22.1	2210	74	5,208,000	0.71	8,550	57.5	10.0	31.0	1.5	0
5	23.0	2390									
8	22.5	2245	73	4,608,000	0.79	8,050	61.5	7.5	28.5	2.5	0
12	24.0	2405									
15	24.0	2365	81	5,376,000	0.76	10,950	51.0	8.0	40.0	1.0	0
19	26.0	2585									
22	25.0	2515	81	5,052,000	0.81	7,150	50.5	3.5	43.0	3.0	0
26	24.0	2375									
29	26.0	2585	82	5,112,000	0.80	16,850	47.0	3.5	45.5	4.0	0
33	26.0	2680									
36	27.0	2680	82	5,312,000	0.77	11,950	42.0	6.0	50.5	1.5	0
40	27.0	2700									
43		2760	82	5,032,000	0.82	7,700	57.0	6.5	35.0	1.5	0
50		2780	93	5,824,000	0.80	12,050	61.5	4.5	33.0	1.0	0
57		2875	97	6,168,000	0.80	11,900	56.5	5.0	34 0	4.5	0
64		3010	96	5,704,000	0.84	10,350	50.5	9.0	39.0	1.5	0
71		2840	98	6,176,000	0.80	10,200	35.5	6.5	56.5	1.5	0
78		2960	92	5,544,000	0.84	11,400	61.5	4 0	32.5	2.0	0
82		2885	95	5,784.000	0.83	8,400	53.0	1.5	41.5	4.0	0
84		2940	98	6,168,000	0.80	10,100	54.0	5.5	38.0	2.5	0

84th day (November 3, 1921) Excellent condition. Sacrificed with chloroform.

TABLE IX

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT OF SILVER-SALVARSAN GIVEN = 0.234 GRAM. TOTAL AMOUNT OF SILVER GIVEN = 33.4 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBIT 21

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	17.4	1733	77	6,384,000	0.61	6,750	63.5	6.5	27.0	3.0	0
5	17.0	1690									
8	17.4	1780	73	5,752,000	0.73	4,450	51.0	4.0	36.5	8.5	0
12	18.6	1865									
15	19.2	1920	88	6,176,000	0.72	7,200	64.0	3.5	30.5	2.0	1
19	19.4	1930									
22	19.8	1970	82	5,744,000	0.72	6,500	68.5	6.5	21.0	4.0	0
26	19.6	1965									
29	20.6	2060	87	5,992,000	0.74	15,200	50.5	5.5	38.0	6.0	0
33	21.0	2100									
36	21.0	2105	91	6,336,000	0.72	8,050	54.5	6.5	32.5	6.5	0
40	22.8	2285									
43		2320	87	6,072,000	0.73	8,950	67.5	3.5	25.0	4.0	0
50		2500	90	6,504,000	0.69	12,750	68.0	5.5	25.0	1.5	0
57		2475	90	6,864,000	0.66	12,100	66.5	5.5	24.5	3.5	0
64		2525	96	6,744,000	0.72	9,250	56.0	7.0	31.0	6.0	0
71		2560	98	6,296,000	0.79	11,600	59.5	3 0	34.5	3.0	0
78		2660	94	6,088,000	0.78	10,300	63.0	3.5	32.5	1.0	0
82		2680	94	6,752,000	0.70	8,550	69.5	2.0	25.0	3.5	0
84		2735	100	6,968,000	0.72	9,800	51.0	8 5	38.0	2.5	0

84th day (November 3, 1921) Excellent condition. Killed with chloroform.

Rabbit 18. Necropsy.—Entirely negative except for a mild degree of liver cirrhosis. A few of the Kupffer cells contain silver granules.

Rabbit 19. Necropsy.—Liver and spleen contain a few silver granules. Kidneys show scattered areas of round cell infiltration. The intervening kidney parenchyma is normal. Bone marrow of femur contains a few groups of silver granules. Necropsy otherwise negative.

Rabbit 20. Necropsy.—Negative except that the liver, spleen and bone marrow contain a very few silver granules. Kidneys show a few scattered scars radially arranged in which there are marked interstitial and parenchymatous alterations. The intervening kidney tissue is normal.

Rabbit 21. Necropsy.—Entirely negative.

DISCUSSION

The experiments yielded no evidence that the repeated intravenous administration of silver-salvarsan into rabbits in doses of 10 milligrams per kilogram (equivalent to a clinical dose of 0.6 gram) or 66.7 milligrams per kilogram (equivalent to a clinical dose of 4 grams) produces any severe tissue alterations.

All of the rabbits that remained healthy and gained in weight during the course of the injections presented no pathologic tissue alterations at necropsy which could be attributed to silver-salvarsan with the exception of the deposit of silver granules found mainly in the liver, spleen, bone marrow, and kidneys. The amount of silver found on histologic examination corresponded very closely with the amounts found chemically after incineration by Myers. The animals that lost in weight showed kidney and liver lesions at necropsy and as a result a much greater percentage of the silver was retained in the tissues.

Before drawing any histopathologic deductions in regard to the tissue changes that may be attributed to the silver-salvarsan, one should first eliminate the pathologic lesions that occur with a certain regularity in the outwardly normal rabbit. In view of the importance of the occurrence of spontaneous lesions in the kidneys and in the livers of rabbits, Doctor Wm. Ophüls, 1910-11, Professor of Pathology, Leland Stanford Junior University School of Medicine, made a careful study of these organs and incidentally of the heart and of the aorta in fifty rabbits. He states that the animals used were partly fresh animals from the market and partly animals raised in the laboratory. He further states that "Several old rabbits that had been in the laboratory for a year or longer were especially selected on account of the greater likelihood of the existence of renal or hepatic lesions in them. Twenty-eight rabbits, among them some of the old ones had entirely normal kidneys, nine showed parenchymatous lesions, three a few areas of cellular infiltration. In ten we found scattered small areas in which were marked interstitial lesions with the formation of small depressions on the surface. Four of these proved to be radially arranged chronic septic foci which extended from the vicinity of the papilla to the outer surface. In the other six cases the same arrangement of the newly formed connective tissue in narrow radial bands which started deep in the pyramids was noticeable although no evidence of

a septic infection could be discovered. The interstitial lesions were accompanied by marked epithelial lesions in two cases only, producing to a certain extent the picture of a chronic parenchymatous nephritis, although the lesions were never very extensive. Lesions of the blood vessels or primary lesions of the glomeruli were not found in any case. Only six normal livers were encountered; the rest showed various stages of coccidial infection with more or less cirrhosis and sometimes cirrhotic processes in which coccidia could not be demonstrated. We are very strongly of the opinion that our findings throw very serious doubt on the numerous reports of the experimental production of cirrhosis in the liver of rabbits as this animal would seem to be entirely unsuitable for such experiments. No gross lesions of the heart or of the aorta were discovered in any of the animals."

Our experience in the routine examinations of apparently normal control rabbits confirms that of Doctor Ophüls. We have also found that a large percentage of the rabbits have spontaneous kidney lesions and that more or less cirrhosis of the liver with round-cell infiltration is the rule rather than the exception. We have further encountered areas of round-cell infiltration in the heart muscle and brain, areas of bronchopneumonia and arteriosclerosis. It is evident therefore that all histopathologic deductions on rabbits should be made with a full knowledge of the occurrence of the spontaneous lesions. This would especially apply to experiments with the arsenic and mercury or bismuth compounds where the drugs are known to produce kidney or liver damage when administered in toxic doses.

The experiments described in this article were undertaken primarily to study the retention and elimination of silver following the repeated intravenous administration of silver-salvarsan. The histopathologic examinations made at necropsy were undertaken with a full knowledge of the spontaneous lesions that frequently occur in the rabbit. The direct object of the histopathologic study was to determine whether silver-salvarsan in the doses administered produced any diffuse kidney lesions or any destructive lesions in the parenchyma cells of the liver and also to determine the sites of distribution of the silver granules. There were no diffuse kidney lesions encountered. The parenchyma cells of the liver were in all cases well preserved.

Our findings are essentially in agreement with those of Kolle, 1918, who states that Professor B. Fischer (Frankfurt a / M.) has determined that rabbits which had received more than 60 injections of 0.07 gram of silver-salvarsan per kilogram intravenously every second day showed no changes of the organs and Professor Goldstein observed also no changes in the nervous system. Symptoms of hypersensitiveness were also not observed in rabbits after injections of silver-salvarsan every second day.

Schlossberger, 1920, states, "In rabbits that received 0.02-0.04 gram silver-salvarsan per kilogram every 3-4 days for 9-12 months, with a total of 3-4 grams, no argyria of the skin could be demonstrated. The silver was deposited in the otherwise normal internal organs, especially in the Kupffer cells of the liver as shown by B. Fischer and to a slight extent also in the

kidneys. No functional injury could be demonstrated for the rabbits, which constantly increased in weight."

In all of our rabbits that received repeated intravenous injections of 66.7 milligrams silver-salvarsan per kilogram the silver granules were deposited in the Kupffer cells of the liver, rarely in the liver cells proper, and frequently in extracellular groups between the liver cell columns and in the periportal connective tissue. The bone marrow tissue in proportion to its size contained even more silver than the liver. The granules were deposited in groups lying free in the tissue and in heavily loaded phagocytes. The spleen pulp contained many free groups of silver granules and a few silver-holding phagocytes. The interstitial tissue of the kidney contained many free silver granules and the tunica propria of the stomach and small intestine contained groups of silver granules and a few silver-holding phagocytes.

In the rabbits that received repeated intravenous injections of 10 milligrams silver-salvarsan per kilogram the silver deposits were inconspicuous. The silver granules were deposited in the sites enumerated above.

The blood examinations were carried out as a preliminary study to determine the effect silver-salvarsan exerts upon the cell elements of the blood. The results are given in detail in Tables II-IX. Blood volume determinations were not made on account of the unsuitability of the rabbit. Anemia was not produced in any of the eight experiments. The majority of the animals showed a gradual increase in hemoglobin and red cells. The white cell counts and differential counts remained within the normal limits of variation.

SUMMARY

Repeated intravenous administration of silver-salvarsan into rabbits in doses of 10 milligrams or 66.7 milligrams every 3-7 days for a period of 47-70 days did not produce any noteworthy tissue or blood alterations.

In the rabbits that received repeated intravenous injections of 66.7 milligrams silver-salvarsan per kilogram (20 times the average clinical dose) many silver granules were deposited in the Kupffer cells of the liver, in extracellular groups between the liver cell columns and in the periportal connective tissue. They were also found in the bone marrow, spleen pulp, interstitial tissue of the kidney and in the tunica propria of the stomach and small intestine.

In a relative way the amount of silver found on histologic examination of the tissues corresponded with the amount found chemically after incineration.

The great individual variation in the amount of silver found at necropsy may have been due to preexisting lesions in the liver and kidneys.

In the rabbits that received 10 milligrams silver-salvarsan per kilogram (3 times the average clinical dose) the silver deposits were inconspicuous.

ACKNOWLEDGMENT

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THE EFFECT OF HIGH CONCENTRATIONS OF ALKALI UPON THE COLOR OF PHENOLSULPHONEPHTHALEIN*

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In the determination of the rate of phenolsulphonephthalein excretion a method is used in this laboratory very similar to that originally described by Rowntree and Geraghty[1]; a standard stock solution is prepared, and a fresh portion of the standard is used in every test by comparing the color produced by the dye in the urine with that of the standard in a colorimeter of the plunger type (Leitz-Dubosq). In preparing the standard solution 1 c.c. of a sterile concentrated solution containing 6 mg. of the mono-sodium salt of phenolsulphonephthalein† is diluted to 2 liters in the presence of an excess of sodium hydroxide. Such a solution is ordinarily used up in about 6 months.

It has been the custom to check the value of each new standard against that of the one previously in use. In almost every instance the results corresponded perfectly; this can be illustrated by the following figures, which are typical of those usually obtained. A solution containing an amount of alkali only slightly in excess of that needed to develop the full color of the dye was prepared on January 8, and this solution had not changed its color, as determined by comparison with a new standard, on June 10. Another solution was prepared on March 3 in which an excess of 10 c.c. of N/10 alkali over that needed to give the full color was used. Since the final volume was 2 liters, this gave an excess concentration of alkali of N/2000. This solution was also checked up on June 10 and showed no change in color. These results are in accord with those described by Rowntree and Geraghty.[1]

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†The solution used was that sold by Hynson, Westcott, and Dunning, for determining the renal function of the kidney.

On one occasion, however, it was thought that the color of a standard solution had faded. A new solution was prepared and it was found that the old solution had lost about half its color in approximately two weeks. Inquiry brought out the fact that a much greater excess of alkali than usual had been used in preparing the poor standard. In order to avoid a repetition of such an error a standard solution containing the least possible excess of alkali over that needed to produce maximum color was prepared. This solution checked satisfactorily against other solutions at the time it was made, and rechecked satisfactorily after it had been in use about two months. Later the color appeared to decrease; the solution was then rechecked, and it was found that it had lost about 25 per cent of its intensity. A further excess of alkali was added to the solution, and the full intensity of the color was restored. It seemed as though the alkali had become neutralized in some way, possibly by exposure to carbon dioxide during the routine use of the solution. These experiences seemed to make it advisable to work out the effect of an excess of alkali upon the rate of fading of the color of phenolsulphonephthalein. It was only when precautions were taken to insure the presence of the least possible excess of sodium hydroxide that fading took place in the presence of low concentrations of alkali, and even then the color did not change until after the solution had been in use for a fairly long time. It seemed to the authors that the addition of too much alkali was more likely to occur and our investigations have been confined to the effect of such additions.

A search of the literature was made for a description of findings similar to those encountered. In a paper by Brightman, Hopfield, Meacham, and Acree[2] published in 1918 a study was made of the endpoints and fading of phenolsulphonephthalein endpoints. They summed up the work of earlier investigators as follows on page 1,940: "* * * phenolsulphonephthalein and other indicators of this series * * * show very little, if any, fading in an *excess* of alkali." The italics occur in the original article. In the experimental part of the work they reported experiments in which 5 to 7 times as much alkali as was necessary to produce a complete change of the compound into its red-purple form were added, and showed by spectrophotometric methods that no fading took place in several days.

It seems probable that the sentence quoted above is intended to apply only to the effect of the presence of a small excess of alkali, for in a paper by Lubs and Acree[3] which was published in 1916 and a reference to which was given in the paper by Brightman et al just cited the following statement occurs on page 2,778. "It (the solution of phenolsulphonephthalein) is yellow in slightly acid solution, pink in stronger acids, and purple in weak alkalies. Stronger alkaline solutions discharge the color, probably through hydration, and the colorless solution becomes colored again on the addition of acids."

In the article by Rowntree and Geraghty[1] in which the use of the dye for the determination of renal function was first described, the following directions for preparing the standard are given on page 598. "The standard

solution used for comparison consists of 3 mg. of phenolsulphonephthalein (or ½ c.c. of the solution used for injection) diluted up to 1 liter and made alkaline by the addition of *only* one or two drops of 25 per cent NaOH solution. This is a beautifully purplish-red solution retaining its intensity for weeks or for an indefinite period." The parenthesis occurs in the original sentence, and the italics have been introduced by the writers of the present article. No more definite descriptions or discussion of the effect of strong alkali upon the dye than these were found.

For studying the effect of alkali upon the fading of the color of phenolsulphonephthalein a concentrated solution of the dye was prepared by diluting 1 c.c. of the stock dye solution (6 mg. of the mono-sodium salt) to 250 c.c. with distilled water. Solutions for the experiments were prepared from this by adding sodium hydroxide to 25 c.c. portions until the maximum color was

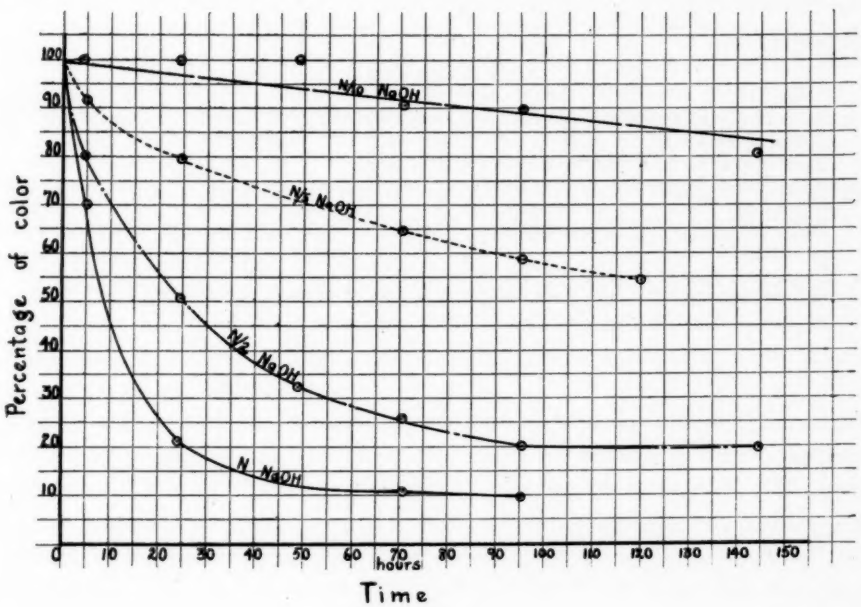

Chart I.

obtained (approximately 11 c.c. of N/100 sodium hydroxide were necessary) and diluting to 200 c.c. in the presence of enough sodium hydroxide to give a final excess concentration of normal, half normal, fifth normal and tenth normal alkali. Each of the solutions thus prepared contained a concentration of the dye equal to that in the standard used in the renal function test. The color of these alkaline solutions was compared from time to time with such standards as had been found satisfactory in the routine work, and with standards freshly prepared from the concentrated stock solution. The results are shown in the chart. The curves show that all of the solutions lost color on standing, and that the rate of the color change was proportional to the excess concentration of alkali present. The relationship between the curves suggests the conclusion that some definite chemical reaction has taken place. Lubs and Acree,[3] as cited above, have suggested that such changes are due

to hydration, and there is nothing in the results reported to refute such a suggestion. We confirmed the statement of these authors that the color is partially restored by the addition of acid to the faded solution, but found that the full color could not be restored in a short period of time by this method.

The experiments reported show that alkali when present in a concentration of N/10 or more destroys the color of phenolsulphonephthalein. The results discussed in the first part of the paper show that sufficient alkali must be used in preparing the solution if changes in its color are to be prevented. From the work as a whole it seems that a final normality of N/1000 to N/2000 alkali is best for maintaining a permanent standard, but it is probably wise to check the strength of the color of the dye from time to time to be sure that no deterioration has taken place. A buffered solution of suitable hydrogen-ion concentration and buffer content might be used advantageously, but is not necessary.

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LABORATORY METHODS

A NEW AND SHORTER METHOD FOR THE DETERMINATION OF UREA AND SUGAR IN THE BLOOD

By B. Gruskin, M.D.,* Chicago, Ill.

WHILE blood chemistry has developed into a very elaborate branch of biologic chemistry and has expanded until it includes the examination of a considerable variety of blood constituents, it nevertheless remains a fact that sugar and urea represent in a good many, if not in most cases, a sufficient diagnostic guide to the clinician. It seemed, therefore, that it would be desirable to devise a simple, reliable procedure for the determination of these two factors. Rapidity, too, is a very important consideration where the volume of work reaches a high level; as indeed is the case in many a hospital, now that a constantly increasing number of clinicians is appreciating the significance of blood chemistry data.

It is with the aims indicated above that the method offered in this paper was devised. In its favor are claimed increased simplicity and rapidity with undiminished accuracy. The method is a new modification and a unique combination of old principles.

As is well known the estimation of urea, especially in the older methods, has been attended by difficulties of one kind or another; special apparatus, troublesome manipulation or a long lapse of time between the start and end of the determination. Some of the more recent modifications which depend on direct nesslerization of the blood filtrate, after incubation with urease, leave much to be desired as regards the tint and transparency of the solutions to be examined in the colorimeter.

The method in brief is as follows: oxalated blood is incubated with specially prepared urease solution. After incubation a protein free filtrate is prepared. One portion of the filtrate is used for the determination of sugar by the usual Folin-Wu technic and another portion is nesslerized directly for the urea nitrogen. It is necessary to prepare the urease under closely specified conditions so that a minimum amount of bean substance is extracted along with the urease. Foreign bean substance consists of reducing sugars which raise the sugar value and materials which interfere to a greater or lesser extent with nesslerization. However, if the directions given below for the preparation of the urease are reasonably closely followed, no serious difficulty will be encountered.

In no case will the blood sugar be found to be high by more than five or

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six milligrams as compared with the regular Folin-Wu method. This is a correction which may be applied if desired; but in the opinion of the author it represents an error which may well be tolerated in routine blood chemistry work, since blood-sugar results are reported as a rule to two significant figures only.

Procedure.—Measure 2 c.c. of blood into a glass tube (6″ × 1″); add 2 c.c. of urease solution (for preparation, see below). Mix by agitating slightly and incubate ten minutes at 45° to 50° C. Treat with 14 c.c. of tenth normal sulphuric acid and mix. Add 2 c.c. of 10 per cent sodium tungstate solution and shake; filter. Two c.c. of the filtrate are measured into a Folin-Wu sugar tube and treated in the usual manner.[1] Five c.c. of the filtrate are measured into a tube graduated at 25 c.c. Distilled water is added to make about 12 or 14 c.c. Ten c.c. of Nessler reagent[2] are now introduced and sufficient water to bring the volume up to 25 c.c. This solution is compared in a colorimeter with a standard which contains 0.075 milligrams of ammonia nitrogen and 0.5 c.c. of the urease solution diluted with water and similarly and simultaneously nesslerized. The result is expressed as milligrams of urea nitrogen per 100 c.c. of blood. This figure multiplied by the factor 2.14 gives milligrams of urea per 100 c.c. of blood.

Comments on the Procedure.—It will be observed that manipulation is reduced to the simplest terms. Distillation or aeration of the formed ammonia is eliminated; the variety of reagents is reduced, and there is no need for standard alkali and acid solutions. A buffer solution for the urease incubation is superfluous, the blood being naturally buffered. The amount of filtrate obtained from 2 c.c. of blood is about 11 c.c., so that, where only a limited amount of blood is available, it would be possible to determine urea and sugar on 1.5 c.c. of blood; whereas, for example, a method like Van Slyke and Cullen's requires 3 c.c. of blood for the determination of urea alone.

Attention should be called to the behavior of the blood during precipitation inasmuch as it differs in one important respect from blood treated by the older method. It seems that the alcohol of the urease solution has the effect of causing the mixture to foam. It is obvious then that foaming cannot be taken as an indication of incomplete precipitation of the blood proteins. If it is desired to ascertain this point before commencing the filtration, it is only necessary to allow the precipitation tube to stand quietly for two or three minutes. The precipitate has a tendency to float toward the top and the color of the liquid beneath is pink in cases where the proteins have been incompletely precipitated. The acidity is then adjusted, in the usual manner, by careful addition of normal sulphuric acid.

Preparation of Urease Solution.—Three grams of permutite* are washed in a 250 c.c. Erlenmeyer flask with 100 c.c. of 2 per cent acetic acid and then successively with two 100 c.c. portions of distilled water. Five grams of powdered jack bean meal† and 100 c.c. of 30 per cent aqueous alcohol are now added and the mixture is gently shaken for not more than ten minutes. It is then immediately poured on a suction filter previously prepared with a

*Central Scientific Company, Chicago.
†Arlington Chemical Co., Yonkers, N. Y.

3-inch Buchner funnel and two layers of No. 2 Whatman filter paper. (The filter should be prepared in advance so as to make it possible to filter immediately after shaking.) If the first 25 c.c. or so of filtrate are turbid they are returned to the filter. The filtrate should be not more than faintly opalescent. The filtrate is finally diluted with its own volume of 30 per cent aqueous alcohol and is kept in the ice box. This urease will keep five or six weeks at ice-box temperature but it is safer not to use a solution that is older than four weeks. As soon as the clear urease solution cools down to approximately 5° C., certain of the more difficultly soluble bean extractives precipitate, thereby rendering the preparation turbid. This turbidity may be disregarded except in the sense that it is necessary to shake before pipetting.

The directions given above are for 150 to 175 c.c. of urease solution. If it is desired to prepare a larger quantity at one time, not only the reagents but the size of the filter, as well, should be increased proportionately. For example, if 450 c.c. of urease solution are to be prepared, instead of 150 c.c., one starts with 9 grams of permutite in a 750 c.c. flask and quantities of bean meal and reagents are trebled throughout. The time of shaking remains the same, ten minutes, and a sufficiently large Buchner funnel is employed so that filtration is complete in two or three minutes. Above all, the combined time of shaking and filtering should not exceed fifteen minutes.

Analytical Data.—Each of a number of blood specimens was divided into three parts. In one part, urea was determined by Van Slyke and Cullen's method. Another part was analyzed for sugar by the regular Folin-Wu pro-

TABLE I

UREA NITROGEN DETERMINATION

Results reported as mg. per 100 c.c. of blood.

SPECIMEN NO.	VAN SLYKE-CULLEN	AUTHOR'S METHOD	DIFFERENCE
1	25 mg.	22 mg.	−3 mg.
2	29 mg.	31 mg.	+2 mg.
3	7 mg.	6 mg.	−1 *
4	22 mg.	22 mg.	0
5	14 mg.	13 mg.	−1 mg.
6	19 mg.	18 mg.	−1 mg.
7	80 mg.	82 mg.	+2 mg.
8	43 mg.	45 mg.	+2 mg.
9	18 mg.	16 mg.	+2 mg.
10	16 mg.	15 mg.	−1 mg.

*This blood was unusually low in urea nitrogen by both methods.

TABLE II

SUGAR DETERMINATION

SPECIMEN NO.	FOLIN-WU	AUTHOR'S METHOD	DIFFERENCE
1	240 mg.	242 mg.	+2 mg.
2	100 mg.	101 mg.	+1 mg.
3	81 mg.	79 mg.	−2 mg.
4	92 mg.	93 mg.	+1 mg.
5	352 mg.	355 mg.	+3 mg.
6	89 mg.	90 mg.	+1 mg.
7	100 mg.	101 mg.	+1 mg.
8	111 mg.	109 mg.	−2 mg.

Results are expressed as milligrams of sugar per 100 c.c. of blood.

cedure. Finally the third part was analyzed for both sugar and urea by the method described in this paper. The results are compared in Tables I and II.

These discrepancies are not greater than found between any two different methods used in blood chemistry or between duplicate results by the same method.

The sugar values recorded above were reduced by five milligrams to correct for the slight sugar content of the urease solution.

It will be seen that the results are all in pretty fair agreement. Since these comparative tests were made, a large number of blood specimens has been analyzed by this method at my own laboratory and in the Pathological Laboratory of the Mount Sinai Hospital, Chicago, Ill. The results have been uniformly satisfactory in all cases.

Note: Acknowledgment is made to my associate, Dr. Harris, for helping me carry out the experimental work.

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A MODIFICATION OF THE KRAMER-TISDALL METHOD FOR THE DETERMINATION OF SODIUM IN BLOOD SERUM*

By Russell L. Haden, M.D., Kansas City, Kansas

THE Kramer-Tisdall method[1] for the determination of sodium in blood serum involves the direct precipitation of the sodium as sodium pyroantimonate, filtration through a Gooch crucible, and weighing. The filtration process has made the method rather uncertain in the hands of other laboratory workers. McVicar and Ross[2] state that: "Even with scrupulous care, we found the method exceedingly temperamental and numerous failures to secure good duplicate results remain unexplained." The technic as originally described requires also the use of platinum dishes for the precipitation, although Wilson[3] has shown that tin dishes may be substituted. The precipitate adheres so closely to glass containers it cannot be quantitatively transferred therefrom.

In attempting to obviate the difficulty and uncertainty of filtration through the Gooch crucible, we first tried fine alundum crucibles as suggested by Dr. Carl H. Green.[4] After a very few filtrations however, the pores of the crucible became clogged and the readings were too high.

We have found that the filtration process may be avoided entirely by the use of the centrifuge and ordinary high form porcelain crucible. A No. 0 crucible will seat well in the top of a 50 c.c. cup of an International centrifuge.

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The precipitation is made directly in the weighed crucible, which is then centrifuged. The supernatant fluid is removed by decantation. By utilizing this procedure we have been able to get accurate check determinations and uniformly good results (Table I). All determinations have been made on 1 c.c. of serum since a crucible larger than No. 0 will not seat in the centrifuge cup. The trunnion ring holding a 50 c.c. cup can be reamed out and a larger crucible be set directly in it, if it be deemed desirable to use 2 c.c. of serum.

TABLE I

RECOVERY OF SODIUM FROM KNOWN SOLUTIONS OF SODIUM CHLORIDE

	CALCULATED MG. PER 100 C.C.	RECOVERED MG. PER 100 C.C.	ERROR PER CENT
	197	193	−2
	197	181	−8
	246	251	2
	246	253	2
	296	316	7
	296	312	5
	346	357	3
	346	369	4
	394	398	1
	394	396	0.5
Aver.	296	302	2

The technic which we have employed is as follows: 1 c.c. of blood serum or plasma is transferred to a weighed No. 0 Coors' high form porcelain crucible. Five c.c. of the potassium pyroantimonate reagent prepared as directed by Kramer and Tisdall is added. Two c.c. of 95 per cent alcohol is then run in drop by drop, the specimen being stirred continuously with a rubber tipped rod. Thorough stirring is very essential. After standing 10 to 15 minutes the crucible is placed in the centrifuge cup and spun for 5 minutes. The speed of the centrifuge should be gradually accelerated. The supernatant fluid is decanted completely, the last drop being removed by touching the rod to the edge of the crucible. Two c.c. of 30 per cent alcohol is then added. This should be allowed to run down the sides of the crucible, care being taken not to disturb the precipitate. The crucible is given a slight rotary motion until a bit of the precipitate spirals up the center. The crucible is again centrifuged and the supernatant fluid removed as before. The washing is repeated, making two washings in all. The crucible is placed in an oven at 110° C. for one hour, allowed to cool in a desiccator and weighed.

CALCULATION.—The weight of the precipitate divided by the factor 0.1108 equals the number of milligrams of sodium per 100 c.c. serum, when 1 c.c. of serum is used for the determination.

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THE USE OF BRILLIANT GREEN-BILE FOR THE COLLECTION AND STUDY OF TYPHOID STOOLS*

By Leon C. Havens, M.D., and Sophie A. Dehler, B.A., Montgomery, Ala.

SPECIAL methods for the isolation of B. typhosus from feces are numerous, and several of them are particularly useful. In all of these, however, attention has been directed to the recovery of the organism from the specimen after it reaches the laboratory, while the value of the specimen has diminished progressively with the time which elapses between its collection and its examination. The specimen must reach the laboratory with the least possible delay to obtain a dependable result, since, with the methods of preserving the specimen usually employed, there is a rapid diminution in the number of viable bacilli of the typhoid-dysentery group.

There are two methods in common use for the collection of such specimens. One is merely to place the feces for the examination in a clean container and start the examination as soon as possible after collection; in other words, have the specimen as fresh as possible. The other method consists in placing the feces in a solution of glycerin (20-30 per cent), which has the advantage of inhibiting B. coli, but also, as will be shown later, has an inhibitory effect on the members of the typhoid group. Consequently it is open to the same objection of increasing unreliability with delay in examination.

An excellent culture medium for typhoid stools would be one which inhibits B. coli and at the same time exerts no deleterious effect upon B. typhosus. The method which is here described accomplishes this result.

DESCRIPTION OF METHOD

Brilliant green is dissolved in bile in a concentration of 1:1500. Difco dehydrated bile is satisfactory and convenient, and has been used in the work described in this paper. After the dye is thoroughly dissolved in the bile it is dispensed into ordinary one ounce wide-mouth bottles, about fifteen c.c. to the bottle, the bottles are stoppered with ordinary corks and sterilized in the autoclave at fifteen pounds for fifteen minutes. After sterilization the mixture keeps indefinitely.

For feces one or two grams are added to the bottle. A larger amount interferes with the action of the bile-dye solution and defeats its purpose.

When the specimen is received at the laboratory a loopful from the surface of the emulsion is plated immediately and this procedure is repeated after twenty-four and forty-eight hours, making three sets of plates from each specimen. The specimen is kept at room temperature in the meantime.

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Three platings on successive days are important, because, as will be shown later, the number of positive specimens is doubled by this procedure. The technic after plating follows the common procedure. The cultures here described were streaked on endo agar, suspicious colonies fished to Russell's double sugar agar, and positive cultures agglutinated with known typhoid serum of high dilution (1:50,000).

EXPERIMENTAL

The value of field studies of typhoid is largely dependent upon the value of single feces cultures. Any method which increases the number of positive findings adds to the fruitfulness of carrier surveys and promotes accurate diagnosis of cases. It is known that bile is a good culture medium for the colon-typhoid group and that brilliant green inhibits B. coli. If this dye exerts the same selective action in bile as in broth a concentration might be found which would permit the use of the bile for the multiplication of the typhoid bacilli originally present in the feces and at the same time prevent the enormous overgrowth of B. coli.

Table I shows the effect of varying concentrations of brilliant green in bile upon pure cultures of B. coli and B. typhosus. Bottles containing 15 c.c. of bile in which the dye was dissolved in concentrations from 1:750-1:3000, were inoculated with 0.1 c.c. of broth cultures of these organisms. The bottles were then kept at ordinary room temperature for twenty-four hours, and 0.1 c.c. of the bile-dye solution plated. The table shows the number of colonies after twenty-four hours incubation in one such experiment, which is typical of all the results.

TABLE I

GROWTH OF B. TYPHOSUS AND B. COLI IN PURE CULTURE IN BRILLIANT GREEN-BILE. INCUBATION OF SPECIMENS AT ROOM TEMPERATURE

CONCENTRATION OF DYE	P_H 6.0		P_H 6.6		P_H 7.2		P_H 7.8	
	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:
1:3000	Innumerable	5000	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	500
1:2400	Innumerable	300	Innumerable	500	Innumerable	300	Innumerable	50
1:1800	Innumerable	100	Innumerable	300	Innumerable	50	Slight inhibition over control	10
1:1500	Innumerable	100	Innumerable	100	Innumerable	15	Slight inhibition	10
1:1000	2000	200	1500	50	2000	10	1000	0
1:750	60	100	50	20	10	12	15	0
Control Bile without dye.	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable

It is seen from Table I that while the selective action of brilliant green on B. coli takes place in bile, much larger amounts of the dye must be used than in broth. Growth of B. coli is inhibited at a concentration of 1:3000 in bile, while inhibition of the typhoid bacillus does not occur until the

dye is used 1:750. The amount of dye used in the method (1:1500) allows a considerable margin of safety on either side, being only one-half the concentration which inhibits B. typhosus and is double that necessary to completely inhibit B. coli in pure culture.

It is further seen from Table I that growth of B. coli is markedly inhibited by all concentrations of brilliant green above 1:3000 at all H-ion concentrations of the bile. But at P_H 7.8 the differential action of the dye appears to be less marked. As one proceeds toward the acid side the inhibitory action on B. coli is less effective. On this basis, we have used the bile-dye solution with the H-ion concentration adjusted between P_H 6.8 to 7.2. If Difco bile is used this adjustment is unnecessary.

Table II indicates an increased inhibitory action of brilliant green in bile on both B. typhosus and B. coli at body temperature. Since the typhoid bacillus grows well at room temperature we have found that better results are obtained by incubating the specimen at 20°-25° C., i.e., in the room, rather than at 37° C.

These experiments, then, indicate that brilliant green dissolved in bile in a concentration of 1:1500 will serve as a useful medium for feces cultures for typhoid. The H-ion concentration should be around P_H 7.0 and the specimen should preferably be allowed to stand at room temperature, because of the apparent increased activity of the dye at 37° C.

TABLE II

GROWTH OF B. TYPHOSUS AND B. COLI IN PURE CULTURE IN BRILLIANT GREEN-BILE INCUBATION OF SPECIMENS AT 37° C.

CONCENTRATION OF DYE	P_H 6.0		P_H 6.6		P_H 7.2		P_H 7.8	
	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:
1:3000	Innumerable	0	Innumerable	Innumerable	5000	5000	5000	1000
1:2400	Innumerable	3	Innumerable	25	5000	30	2000	200
1:2100	Slight inhibition	0	Innumerable	0	1000	0	5000	10
1:1800	1000	10	Innumerable	50	500	0	1000	50
1:1500	500	5	Slight inhibition	50	100	0	5000	100
1:1250	50	0	500	10	10	0	500	10
Control Bile without dye	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable

These facts were arrived at with the use of pure cultures. In order to determine its effectiveness for typhoid stools, known numbers of B. typhosus were added to specimens of feces received at the laboratory for other examinations. At the same time part of the same specimen of feces containing the same number of typhoid bacilli was introduced into a bottle of 20 per cent glycerin for comparative results.

It is seen in Table III that there is a striking increase in the recovery of B. typhosus from feces in the brilliant green-bile as compared with glycerin

TABLE III

RECOVERY OF KNOWN NUMBERS OF B. TYPHOSUS FROM FECES IN 20 PER CENT GLYCERIN AND BRILLIANT GREEN-BILE

NO. B. TYPHOSUS ADDED PER GM. FECES	TOTAL SPECIMENS	GLYCERIN 20 PER CENT SPECIMENS POSITIVE				BRILLIANT GREEN-BILE SPECIMENS POSITIVE			
		24 HR.		48 HR.		24 HR.		48 HR.	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
500-2000	19	19	100	9	47	19	100	19	100
50- 100	47	14	30	5	11	37	79	43	91
10- 20	27	2	8	1	4	23	85	24	88
2- 5	7	0	0	0	0	6	85	4	57
TOTAL	100	35	35	15	15	85	85	90	90

solutions. In fact, in six of seven samples, as few as three or four typhoid bacilli were recovered from feces in the bile-dye solution in forty-eight hours, while none could be recovered from glycerin. The experiments summarized in Table III further show that no deterioration of the specimen takes place up to forty-eight hours in the bile-dye solution since the percentage of positive results is slightly higher than at the end of twenty-four hours. The same samples in glycerin, on the other hand, show a progressive decrease in the number of viable typhoid bacilli. The efficiency of the two methods is indicated by the total number of recoveries in each case. With glycerin this was 35 per cent and 15 per cent at the end of twenty-four hours and forty-eight hours respectively, while with the bile-dye solution, it was 85 per cent and 90 per cent. Reference to Table V shows that the typhoid bacillus continues to multiply for seventy-two hours, or longer, since the number of positive results was doubled by plating on the third day.

APPLICATION

It is evident from the foregoing experimental evidence that brilliant green-bile permits the recovery of small numbers of typhoid bacilli from feces. Since the majority of the specimens received at a state laboratory come by mail from considerable distances it is important to have a dependable method which minimizes deterioration due to the age of the specimen. The results obtained with such specimens constitute a severe test of the method. Table IV summarizes these results. Of 241 specimens, typhoid bacilli were isolated from 71 or 29 per cent. These specimens represent single samples from suspected cases of typhoid fever, and from convalescent and chronic carriers. Epidemiologic studies of a number of small rural outbreaks are responsible for the relatively large number of positive carrier cultures.

TABLE IV

RESULTS OF FECES EXAMINATIONS RECEIVED IN BRILLIANT GREEN-BILE

	TOTAL	NO. POSITIVE	PER CENT POSITIVE
Suspected Carriers	126	14	11.1
Suspected cases	115	57	49.5
Total	241	71	29.4

Positive findings in practically one-half of all single specimens sent by mail from suspected cases of typhoid bespeaks a high degree of efficiency for the method used. Since a considerable number of these cases were only suspected to be typhoid more than 50 per cent recoveries were obtained in this series with a single specimen in proved cases.

The results summarized in Tables III and IV give evidence that the usual rapid diminution of typhoid bacilli in feces is prevented by suspension of the specimen in brilliant green-bile. Table V adds further evidence of the truth of this statement.

TABLE V

RESULTS OF EXAMINATIONS OF FIELD SPECIMENS ON SUCCESSIVE DAYS

	24 HOURS	48 HOURS	72 HOURS	TOTAL
Number Positive	34	57	68	71

Table V further shows that if the specimens had been plated only on the day of arrival at the laboratory more than half of the positives would have been missed. If they were to be plated only once the third day is apparently the best time, but it is the routine procedure in this laboratory to plate on three successive days. An increased number of platings, in itself, increases the chances of a positive result.

DISCUSSION

The typhoid bacillus begins to die in feces immediately and, in fact, its numbers progressively diminish in the intestinal tract after leaving the duodenum. This fact has necessitated the use for the recovery of the typhoid bacillus of the fresh feces or of some method of preservation. Successive platings in the laboratory have been valueless for the same reason. A method which permits the organisms of the typhoid group to multiply, not only enhances the value of the initial plating, but permits the use of repeated examinations with increased chances of success. Such a method is especially valuable in a state laboratory where delayed examinations are often necessary. Furthermore the likelihood of obtaining a positive culture from a single specimen is increased, since, even though the bacilli may be few in number in the original sample of feces taken, growth takes place and by the third plating the number of organisms has increased many times. The progressive diminution of the colon bacillus is a further factor in increasing positive results.

The experimental work described in this paper deals with B. typhosus. The method however, has been used for paratyphoid A and B and the dysentery bacillus, with equal success. In other words, the method is useful for all members of this group. The bile-dye solution has also been used with success for blood and urine cultures.

It is needless to point out the value of such a culture medium in field investigations of typhoid outbreaks. Such investigations must frequently be carried out at a considerable distance from a laboratory and it is not always

possible to obtain more than a single specimen from any one individual. Anything which adds to the value of the result of a single specimen is a material aid to the epidemiologist.

In a typhoid carrier survey now being conducted among food handlers the results so far indicate that a rate of approximately 2 per cent will be obtained with this method. The results of this investigation will be made the subject of a future paper.

SUMMARY

A solution of brilliant green in bile will inhibit B. coli and yet allow B. typhosus to multiply. This principle has been utilized successfully in the culture of feces for typhoid. It overcomes the loss of dependability resulting from delayed examination and permits recovery of the typhoid bacillus from the specimen even after several days.

THE USE OF AUTOGENOUS VACCINES IN PULMONARY DISEASES WITH SPECIAL REFERENCE TO THE COHEN-HEIST METHOD OF PREPARATION*

By J. H. Clark, M.D., Philadelphia, Pa.

IT is only within the past ten or twelve years that vaccines have been used to any extent in the treatment of pulmonary infections. Gillett[1] and Pirie[2] in England, and Babcock[3] in this country reported favorably upon the use of autogenous vaccines in chronic bronchitis and asthma. Scheffegrell[4] has employed a mixed treatment with vaccines and pollens in hay-fever with benefit, while Walker,[5] and Hutcheson and Budd,[6] reported favorable results from vaccine therapy in cases of bacterial asthma. Many of the earlier failures were undoubtedly due to the placing of too much reliance on vaccines alone and not considering them as adjuvant to treatment. Within recent years their scope, in pulmonary infections, has increased greatly and many conditions that were formerly treated by "watchful waiting" and unsuccessful surgical methods, have been successfully cured by vaccine therapy.

In a cursory review of recent literature, one is struck with the variety of methods of administration of vaccines, their preparation and the diversity of conditions in which they are used. Minet[7] employed autovaccines in chronic conditions and stock in acute. The sputum was cultured on three successive days, rough estimates of the different organisms present were made and an average of the three days computed. This average formed the basis of the autogenous vaccine. Noting that the bacterial flora in both the acute and chronic conditions was practically constant, he discarded this laborious

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method of preparation and later used stock vaccines solely, based on his previous bacteriologic findings. The stock vaccine for chronic conditions consisted of 20 million pneumococci, 40 million tetrageni, 40 million micrococcus catarrhalis and 400 million staphylococci per c.c. This was given every two days, with a rest of five or six days between the fifth and sixth injection, until ten doses in all had been given. In chronic conditions secondary to tuberculosis, he found neither febrile reaction, nor hemoptysis, but noticed a diminution in the cough and improvement in the general health. It was also beneficial in chronic bronchitis. His stock vaccine for acute respiratory conditions was composed of 100 million pneumococci, 50 million streptococci and 350 million staphylococci per c.c. One c.c. was given every two days, dropping to 0.5 c.c. if the heart or kidneys were affected. It is interesting to note that healing was obtained by brutal shock in the acute conditions; if there was no shock there was no healing. This evidently borders on the nonspecific (protein shock) reaction that has been utilized, at times, in typhoid fever and pneumonia. Later[8] he claimed remarkable cures in asthma, using the same stock vaccine for chronic conditions; he again noted a diminution in the quantity and purulent character of the sputum and improvement in the general health of the patients with pulmonary tuberculosis; he cured one-third of his cases of emphysema and catarrhal bronchitis, improved markedly another third and considered an absence of betterment unusual.

The favorable results obtained by Minet influenced other observers to employ stock vaccines in both acute and chronic pulmonary infections. Bourges and Jabard[9] report a case of emphysema and catarrhal bronchitis with recurrent asthmatic attacks of several years standing, in which dyspnea, even on exertion, disappeared after the sixth injection of Minet's stock vaccine, injected every two days. Three months later all auscultatory signs and râles had disappeared and there was no recurrence of asthmatic attacks. These authors also report, as an instance of vaccine therapy, a case of lobar pneumonia, in which 1 c.c. of Minet's stock vaccine for acute respiratory infections was given on the fourth day of the disease. Recovery followed a severe chill, sweat and initial rise of temperature, which later fell to normal and stayed there.

Lambert[10] reports cases illustrating the efficacy of vaccines in suppurative conditions of the pleura. In one, a case of an old purulent pleurisy, with fistula following operation, the cavity was made sterile after ten injections of an autogenous vaccine and closed by secondary suture. A second case of an old revolver wound of the chest, was cured by vaccine therapy alone, although two vaccines were necessary, a procedure that is often neglected in old indolent conditions. DeVernejoul[11] feels that in suppurative conditions of the lungs, surgical treatment should be completed by vaccine therapy and preferably autogenous vaccine therapy.

Abadie and Dufau[12] report a case of abscess of the lung with gangrene, developing after a typical attack of typhus fever, cured by autovaccine. The sputum contained streptococci, staphylococci, Friedländer's bacillus and B. proteus vulgaris. An autogenous vaccine made from these was given at

three day intervals and after the seventh injection the patient was discharged completely cured on the twenty-fourth day.

Dufour and Ferrier[13] emphasize that if vaccines are not efficacious in the early acute case, they may help in those becoming subacute to chronic, confirming their opinion by reporting a case of pleurisy and pulmonary congestion, with fetid sputum, which was cured by autogenous vaccine.

In this day of nonspecific therapy, one hesitates to advocate too strongly the employment of specific bacterial vaccines. Kolmer[14] says, "The treatment of disease by the administration of nonspecific agents has attracted a great deal of attention during the past few years. Indeed, it would appear that this form of therapy is in danger of being overused and its importance overemphasized." It is especially beneficial in bacterial infections due probably to an increase of congestion about the foci of bacteria and absorption of the bacterial products, leading to an autoimmunization and hence specific stimulation. But does it not seem probable, if nonspecific substances will increase immunity against a definite organism or organisms, by specific stimulation, that specific, definite, bacterial vaccines will be even more efficacious?

Assuming, for the moment, that the use of specific vaccines is just as beneficial as nonspecific, for a certain degree of success in vaccine therapy is probably due to both effects, what type or types of definite bacterial vaccines are considered to be the better, stock or autogenous? For prophylactic purposes stock vaccines are probably to be preferred (Jenkins[15]) or in the treatment of acute pulmonary infections (Minet[16]), but most writers agree that autogenous vaccines, i.e., those prepared from the patient's own bacteria, should be used whenever possible, especially in the vaccine treatment of disease.

Autogenous vaccines to be successful should contain, preferably, the definite and determined organism or organisms, which are responsible for the condition and are not simply saprophytic. It is comparatively simple to grow bacteria from the discharge from an infected area, suspend them in phenolized saline, kill by heating, test for sterility and tell the physician to use so much. But how does one know these are the offending organisms? There are different methods of determining which bacteria are etiologic or pathogenic and which are saprophytic. One method is by agglutination. Walker[17] prepared dried bacterial allergens by repeated centrifuging with saline, alcohol and ether and performed skin sensitization tests with the same. Kolmer[18] employs intracutaneous tests with bacterial suspensions; or one can test the bactericidal activity of the blood by the Lacy-Heist method.[19] All of these methods, however, require more apparatus, simple though it may be and are time-consuming.

Wright[20] working in South Africa on pneumonia, noted that blood flowing from capillaries had bactericidal activity not present in defibrinated, or citrated blood or serum. Cohen and Heist,[21] using the Lacy-Heist method, stated that the bactericidal activity of coagulable blood offered a means of determining natural or acquired immunity. They emphasized[22] the importance of using "unaltered, coagulable, whole, flowing or living blood." Later

Cohen[23] suggested the possibility of separating bacteria, that are etiologic and infecting, from those that are merely saprophytic and nonpathogenic, in copious discharges containing many types of organisms, by growing the infected material directly in whole blood. For in working with Heist and later Borrow, he had determined that, "blood of human beings possesses bactericidal power against large numbers of organisms; that the blood of an individual differs in its bactericidal power against different organisms; that bactericidal power against a particular organism varies in different individuals; that in the discharge from an infected area, organisms can usually be found, against which the blood of the infected person has little or no bactericidal power; that frequently in such discharges or on such area other organisms are found against which the patient's blood has good bactericidal power; that organisms that are supposed to grow well in human blood, fail to grow at all in the blood of some individuals; and that organisms that are supposed to grow poorly or not at all in human blood, may grow with the greatest vigor in the blood of other individuals." It was felt that organisms growing in whole blood was strong presumptive evidence that they were responsible, either as an etiologic or complicating factor, and that when no growth was obtained, they were probably saprophytic. This led to growing the infected material directly in whole blood, plating and identifying the organisms and making a vaccine from the growth so obtained. It had the distinct advantage of saving time and presumably finding the offending organism by a single simple procedure. The method consisted in merely coating the bottom of a sterile test tube with some of the discharge, collecting whole blood aseptically and placing it immediately in the test tube and incubating. Contaminating or nonpathogenic bacteria usually disappeared when grown in the whole blood of the patient studied and as a general rule pure cultures of a single organism were obtained. Cohen and Heist[24] reported several cases of different types of infection successfully treated by autogenous vaccines prepared in this manner, but were conservative in the conclusions drawn, one of which stated, "when an organism present in the respiratory passages, sputum or feces grows in the whole blood of an infected individual, there is a probability that it may be the etiologic or complicating organism."

In our work in connection with the asthma and hay-fever clinic at the Samaritan Hospital, we were disagreeably impressed with the time-consuming method of plating and fishing colonies in preparing vaccines and often disappointed in the results obtained, even though sensitization tests were performed in some cases and vaccines made from the organisms causing positive reactions. The Cohen-Heist method of growing offending bacteria appealed to us as time saving and extremely simple and seemed to do away with the necessity of sensitization tests. In several cases now, we have used the Cohen-Heist method with gratifying results, only a few of which will be reported. In preparing vaccines from sputum we have modified somewhat the original technic. Specimens of sputum are collected after the mouth has been washed out with a mild antiseptic solution. The sputum is then washed in a few changes of sterile saline and a small quantity placed in the bottom of a

sterile test tube. This is covered with about five cubic centimeters of blood freshly drawn from the individual, collected in a sterile syringe with the usual aseptic precautions. The whole is incubated for eighteen to twenty-four hours. No attempt is made at identification, beyond morphology and staining characteristics. The growths obtained have been, with one exception, single pure cultures (Case 3). Nor have there been any instances of contamination with the usual laboratory contaminants.

CASE 1.—Young adult male. Gave a history of having been gassed in the war. Had complained since then of loss of weight, persistent cough and a slightly fetid sputum, moderate in amount. A vaccine was prepared by the Cohen-Heist method, yielding a Streptococcus. Injections were given at four day intervals with only slight local reactions. After five weeks the patient had gained eleven pounds in weight. The disagreeable odor of the sputum had disappeared and cough practically ceased.

CASE 2.—Male aged forty. Following a submucous resection and tonsillectomy, there developed fever, cough and copious fetid foul-smelling sputum, offensive and almost unbearable to patient and family alike. X-ray confirmed the clinical diagnosis of pulmonary abscess. An autogenous vaccine by the Cohen-Heist method gave a large gram positive coccus. Injections were given at four day intervals, with practically no local reaction. After three injections the sputum diminished in amount and lost much of its former offensive odor and the general health improved. A few weeks later a second vaccine was made in the same way from a gram positive coccus. Artificial pneumothorax was performed and a cure effected together with the second autogenous vaccine. The patient has gained forty-five pounds in weight and is back at work.

CASE 3.—Male aged sixty-six. Has suffered for the past twenty-two years with chronic bronchitis and bronchiectasis. Sputum almost one quart daily. Vaccine was attempted in this case solely as a palliative measure to try to diminish the quantity of sputum. The Cohen-Heist method yielded a Streptococcus and Staphylococcus. The sputum has decreased in amount to about a half-pint. The cough during the day has almost entirely disappeared, but there are still paroxysms in the morning until the bronchiectatic cavities have been emptied of the secretion accumulated during the night. The patient has returned to work after six months absence.

CASE 4.—Male aged forty-eight. For the past fifteen years has suffered from chronic bronchitis and asthmatic attacks. The usual sensitization tests had yielded negative results. Culture by the Cohen-Heist method gave a Streptococcus. It is now four months since the vaccine treatment has ended. The cough has entirely disappeared and benzyl benzoate has controlled the asthmatic attacks.

These four cases illustrate the kind of result we have obtained by autogenous vaccine therapy in pulmonary infections. Our experience is not large, nor are all cases entirely successful. We know in certain suppurative conditions, the results obtained by autogenous vaccines are striking, even when used alone. We feel in other conditions, that they form a distinctly beneficial adjuvant to treatment. We submit the Cohen-Heist method of preparation, as it lends itself as a rapid and simple means of preparing an autogenous vaccine, offering, in addition, the detection of organisms against which the individual has no demonstrable immunity.

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THE USE OF ACETYLENE GAS FOR HEMOGLOBIN ESTIMATION*

By H. R. Miller, M.D., New York City

It is well known that carbon monoxide as it passes through fluid blood combines with hemoglobin to form carbon monoxide hemoglobin. Similarly nitric oxide, under the same circumstances, forms nitric oxide hemoglobin. Both these products are stable.

In 1892 Hoppe-Seyler[1] reported a method for hemoglobin estimation based upon the knowledge that hemoglobin and carbon monoxide enter into a permanent union.

In 1901 Haldane[2] revised the method, employing Gower's hemoglobinometer. For a standard he used 1 per cent ox or sheep blood having an oxygen capacity of 18.5 volume per cent, saturating the blood with carbon monoxide. The blood to be tested is laked, bubbled with illuminating gas and matched against a known standard.

Palmer[3] in 1918 further modified the method. Human or ox blood of an oxygen capacity of 18.5 volume per cent, is defibrinated. The oxygen capacity is determined by the Van Slyke method. A 20 per cent solution of blood is made with 0.4 per cent ammonium hydroxide, through which illuminating gas is passed for ten minutes, then carefully sealed and preserved as a standard. To carry out a hemoglobin determination of unknown blood a 1 per cent solution of unknown blood is laked in 0.4 per cent ammonium hydroxide solution, and then saturated with illuminating gas. It is matched in a colorimeter against a standard consisting of a 1 per cent solution freshly prepared from the original 20 per cent standard and newly resaturated with illuminating gas. The standard is set at 10. The calculation is made by dividing the known by the unknown and multiplying by 100. This method is trustworthy and accurate.

Carbon monoxide is not always on hand, especially for bedside studies,

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and for many occasions it may be inconvenient or impractical to employ it. I have, therefore, substituted acetylene gas for carbon monoxide. This gas readily unites with hemoglobin to form a combination almost as permanent as carbon monoxide hemoglobin. It has the advantages of being readily produced, of being less toxic and disagreeable when used in ordinary amounts in an open room. I have found it convenient to obtain acetylene gas by dropping a very small piece of calcium carbide (about 50 mg.) into an ordinary 15 c.c. test tube half full of tap water. Gas is generated immediately, the tube is quickly stopped with a rubber plug through which a tube con-

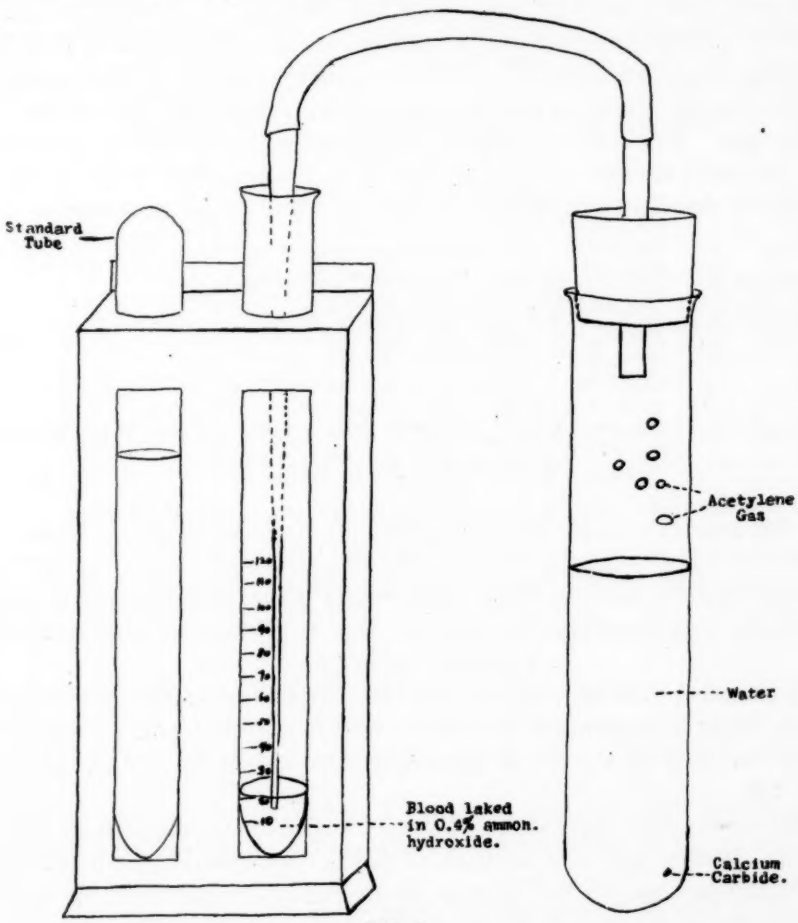

Fig. 1.

nects with a piece of rubber tubing fitted over a narrow capillary pipette (see illustration). The capillary tube may now be led into the solution of blood ready for saturation. A few seconds of bubbling will cause a change to a pink color typical of acetylene hemoglobin.

The use of acetylene gas in hemoglobin estimation has proved practical for the Palmer and Sahli methods. The Palmer method represents a simplification of the Hoppe-Seyler and Haldane principles. Acetylene can be substituted for carbon monoxide. The original 20 per cent standard is prepared with acetylene gas; stored in the dark and on ice it will keep for several

months. As yet we have had no opportunity to test its keeping quality beyond nine weeks.

The use of acetylene gas is also applicable in the estimation of hemoglobin by the Sahli hemoglobinometer. Two millimeters of freshly drawn blood are raised into an accurately gauged Sahli pipette and promptly emptied into 0.4 per cent ammonium hydroxide solution previously poured to the 10 mark into the calibrated Sahli tube. Into this tube acetylene gas is led for a few seconds through a fine capillary pipette, taking care not to raise the fluid by bubbles onto the sides of the tube. Ammonium hydroxide solution (0.4 per cent) is now added drop by drop until the solution is matched in the Sahli instrument against the acetylene treated standard. The standard is prepared by treating 2 mm. of blood of a known oxygen capacity of 18.5 volume per cent with 0.4 per cent ammonium hydroxide, saturating the solution with acetylene gas. This tube is covered with sterile oil, or better, hermetically sealed. A small amount of toluene added to the standard helps preserve it. When not in use it should be kept in the dark and on ice if possible.

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THE USE OF ULTRA-VIOLET RAYS FOR THE POLYCHROMIZATION OF METHYLENE BLUE*

By Stanley U. Marie and Joseph T. Raleigh, Boston, Mass.

THE ultra-violet ray has been found by us to be very efficient in bringing about the polychroming of methylene blue in connection with Romanowsky stains.

Comparative tests have shown that the preparation of Romanowsky stains, in which the polychroming of methylene blue is required, can be accomplished in much less time by the use of ultra-violet rays than by any of the methods now in use.

The method used by us is as follows: Dissolve 1 gram of methylene blue in 100 c.c. of a 0.5 per cent solution of sodium bicarbonate in distilled water. The mixture is exposed to the rays of any ordinary arc lamp or any other lamp capable of supplying the ultra-violet ray in appreciable quantities for from ten to thirty minutes, depending on the amount and depth of methylene blue mixture, also the type of lamp. A shallow dish five inches in diameter is to be recommended. When the mixture is cool, add slowly without filtering 500 c.c. of a 0.1 per cent yellowish eosin solution until the mixture loses its blue color and becomes purplish with a metallic luster on the surface in which a fine black precipitate is formed. Filter this precipitate on paper and allow it to dry spontaneously. One-tenth of a gram of this dry powder is dissolved in 60 c.c. of acetone free methyl alcohol.

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EDITORIALS

Research

IT falls to the lot of few indeed to make a complete investigation of any problem or to carry it to the uttermost boundaries of its eventual possibilities.

The final solution of scientific problems *de novo et in toto* is more often the result of the work of many than the fruition of the labors of one. Just as a crystal reflecting light from a myriad of facets will vary in brilliance in accordance with the angle from which it is viewed, so will a scientific problem present a varying angle of attack in accordance with the viewpoint of the observer. One carries his work, perhaps, to what appears to be a point of completion; another, following, advances the study still another step. Like the formation of a mosaic, each bit of work ultimately fits in somewhere in the great design which, in essence, is the work of many rather than the expression of the completed purpose of one.

Research, scientific investigation, the patient inquiry and the laborious acquisition of information are often, if one does not pause to think, held to be the prerogative of the professional investigator and to connote a vast expanse of laboratories with their association of equipment, expense budgets and so on.

All these, of course, render the worker's path more easy and his opportunities more accessible but the essential prerequisite for scientific investigation lies in the possession of imagination and the power of acute observation coupled with an infinite capacity for taking pains—which is a definition that someone has given of genius.

Laennec discovered the principle of the stethoscope with no greater equipment than a cardboard tube—but observation and imagination preceded and accompanied his discovery without which, indeed, it would not have been made.

Every man thoroughly and actively interested in the practice of medicine is and should be a potential investigator and research worker. Textbooks of medicine, surgery, diagnosis, therapeutics—what you will—are, in essence, the compiled and correlated individual observations of the multitude. The classical and "typical" descriptions of disease are merely the sum total of a host of "lowly case reports" gathered in serried array; new methods of treatment often have their origin in the imagination of single observers; their final evolution and evaluation is more often the sum total of the labors of many.

The study of carefully kept case records; attention to and cogitation over what in themselves are minutiae with an investigation of what imagination suggests as their possible ultimate significance is just as truly research as an abstruse investigation secluded within the walls of spacious laboratories.

"Theory," says Hippocrates, "is the flower, not the root of experience."

"Put off your imagination as you take off your overcoat when you enter the laboratory," says Claude Bernard, "but put it on again as you do your overcoat when you leave the laboratory. Before the experiment and between whiles, let your imagination wrap you around; put it right away from you during the experiment itself lest it hinder your observant power"—thoughts as applicable to clinical as to laboratory studies.

Every physician should be an investigator, a research worker in essence even if his studies enlighten no one but himself, but if to keen observation and careful thought he couples a capacity for taking infinite pains, he cannot avoid some addition, however minor, to the sum total of medical knowledge.

The clinician and the investigator are one in their object. The labors of one are assisted, corroborated, proved or disproved and finally evaluated by the aid of the other. Clinical and laboratory medicine are not separate entities but inseparable associations and a part of one whole—which is the thought behind the name and *raison d'être* of this journal.—*R. A. K.*

The Evolution of a "Standard" Method of Complement Fixation in Syphilis

AFTER nearly twenty years of painstaking effort the complement-fixation test has come to occupy a definite and fairly well defined place in the clinical and laboratory study of syphilis and, in the interest of uniform and strictly comparable results, the attention of serologists and clinicians is now focussed upon the problem of evolving a technic acceptable for uniform adoption.

The problem has been studied by various investigators and groups from various angles but while much has been done and various methods of performing the test proposed, their general adoption or even wide-spread trial has not followed for reasons which need not be elaborated.

It is rather apparent that, to secure its general adoption, the standard method, if it be not the joint product of a delegated group, must have, to secure its general trial and eventual adoption, the accolade of approval officially or semi-officially conferred upon it in so unmistakable a manner that it will be impossible for the conscientious worker to ignore it on any grounds.

Service or cooperation with such a group engaged in such a problem should be considered a public duty and there already exist two representative bodies of laboratory and clinical workers preeminently fitted to conduct such a survey, one of which has already manifested a collective interest in the problem: The American Public Health Association, and the American Society of Clinical Pathologists.

The personnel of either—or better, of both—of these organizations furnishes an unimpeachable forum for the presentation and discussion of ideas and a representative body by which investigations may be conducted. If a consensus of agreement can be reached by either or both in conjunction, its tenor will be sufficiently representative and authoritative to cause it to be heard and respected.

In order to avoid unnecessary repetition of completed investigations whose accuracy and significance is accepted, an important preliminary to such an investigation, it might be suggested, would be the collection and analysis of opinion as to the essential desiderata to be incorporated in a standard technic and from which the specifications of such a technic could be constructed.

Next in order, because of the difficulty of securing a general acceptance of methods with which a certain proportion of workers may be more or less unfamiliar and in favor of which, accordingly, they are apt to be reluctant to cast aside those well tried by time and personal experience; and in order to avoid the impression of personal bias in the investigation it would seem logical as well as feasible to select from those methods in the field at large, those which claim or appear to most nearly approximate the requirements for a standard technic.

By apportioning these for comparative trial among the personnel of the investigating bodies it should then be easy to secure within a short space of time a sufficiently large number of comparative tests without unduly burdening any worker or laboratory.

One thousand tests by one hundred workers gives a total of one hundred thousand reactions for comparison and analysis in much less time than would be possible for one and, in addition, the personal factor is minimized in their analysis.

By the same method when, at any time, an apparently irreconcilable difference of opinion arises in the consideration of essential desiderata, its thorough comparative investigation is rapidly attained.

If, at the end of such a comparative trial, the field can be narrowed down to one or two or three methods which excel preeminently, the further investigation may be concentrated upon these in the endeavor to see which is the most satisfactory and adaptable to the standard specifications; if none, then the construction of a new technic is indicated and the pathway toward it cleared of many obstacles.

Coincidently with the conduct of such an investigation and analytic survey of the whole, a similar collective study of the parts may be made with reference to the acceptable standard for reagents and so on.

It is much more likely that the *adoption* of such a proposed standard method—which, after all, is the crux of the problem—would more rapidly follow such a collective and authoritative report, than as a sequence to the work of individuals or individual groups.

Even if such a collective investigation should fail at the first attempt, to evolve a perfectly satisfactory and acceptable standard method, it could not fail to finally rid the field of its present impedimenta of loosely constructed methods which, it must be admitted, abound in general practice.

It is not to be assumed, nor would it be tolerated, that any method by whomsoever proposed, should constitute the final and irrevocable solution of the problems inherent in complement-fixation tests. Investigations as to the mechanism and significance of this phenomenon should and would, undoubtedly, continue but their results should be more readily approximated for study and analysis when the methods whereby they were obtained are more strictly comparable than they are at present. —*R. A. K.*

The Gorgas Memorial

DURING the past year, throughout the United States, the work of organizing the Gorgas Memorial State Governing Committees has been progressing. In some states the response has been most enthusiastic, while in others considerable effort has been necessary to bring home to the doctors the importance of this movement to them, individually and collectively. Inasmuch as the Gorgas Memorial is primarily a medical movement and as such must have the united support of the profession if it is to make the proper impression on the general public, we take this occasion to outline briefly the Gorgas plan and to request the cooperation of our colleagues in bringing to a successful issue this national health program.

We are planning to establish a memorial for our former chief, Major General William Crawford Gorgas, not of marble or bronze, but a permanent

living organization in the form of a great health foundation typical of his work in research and curative medicine, that will unite laymen and doctors in an intelligent effort to obtain better personal health—a health guild that will be supported and directed by the representatives of curative medicine.

The Gorgas Memorial consists of two phases:

1. An Institute in Panama for research in tropical diseases.
2. A health educational program in the United States and other countries that wish to cooperate and participate in the movement.

We are living in an age when people are knocking at all doors of knowledge and demanding that they be admitted. In the field of medicine who are so well fitted to meet this demand as those actually engaged in the practice of medicine? The doctors have a far more interesting and important message to deliver than any other group.

In the United States today there is scarcely a community that has not its quota of irregular "medical practitioners," so-called. In many states there are strong organizations of the representatives of the various cults, whose theories are imposed upon an uninformed public. Public ignorance is encouraged by professional reticence and the result is the astounding growth of unscientific methods. If the profession is to maintain the high standing to which centuries of labor in behalf of suffering mankind entitles it, it is essential that a definite organized effort be made to familiarize the public with such facts as will impress upon it the importance of medicine's contributions to human welfare. A constant fund of proper health information through the newspapers, magazines, lectures, moving pictures and the radio, furnished by medical men and women of known reputation and standing, will direct the public to the proper source for medical advice and gradually eliminate the irregular practices constantly increasing.

One of the objects of the Gorgas Memorial is to furnish a channel through which this kind of information may be disseminated. It cannot be done by individual physicians. It must be conducted by a dignified, ethical organization, controlled by the medical profession. The name of Gorgas is synonymous with "better health." No more appropriate name could be adopted for a movement that has for its object, *the development of cooperation between the public and scientific medicine for the purpose of improving health conditions by implanting the idea in the mind of every individual that scientific medicine is the real authority in all health matters and as such should be recognized as the source of health instruction.*

Before we ask the public for financial and moral support, it is essential that the doctors of the country unite in support of this program. As a means to this end, Governing Committees are now in process of organization, on the basis of 100 members to every 1,000,000 population in each state. Seventy-five per cent of the personnel of each committee will consist of medical men and 25 per cent of influential laymen and women. The permanent activities of the organization will be supervised by these committees in their respective states, in cooperation with the National Executive Committees.

An organization cannot operate without funds. We are endeavoring to raise an endowment of $5,000,000, the interest only of which will be utilized to carry on the work. The principal will be invested in trust securities and remain intact. None of the money thus obtained will be spent for buildings or equipment. The Republic of Panama has donated the site and guaranteed the initial buildings and equipment for the tropical research laboratories, in recognition of Gorgas' great work in Panama. Those invited to serve as Founder members of the State Governing Committees are requested, as they accept membership on the Committee, to subscribe $100 to the Endowment Fund, payable within two years. Every individual on the State Committee is a contributing member. When the medical nucleus of the organization is complete, a general appeal for funds will be made to the public.

The American Medical Association, at its recent meeting in Chicago, passed the following resolution:

> "RESOLVED, That the House of Delegates of the American Medical Association, convinced of the great promise which the Gorgas Memorial contains of benefit to humanity through improved knowledge of preventive medicine and tropical disease, and of its peculiar adequacy, as a tribute to our great leader and sanitarian, recommend to the organized profession of the country, through its constituent state and county societies, the enthusiastic support of the project."
>
> J. A. Witherspoon, Tennessee,
> Joseph Rilus Eastman, Indiana,
> Thomas Cullen, Maryland,
> W. H. Mayer, Pennsylvania,
> F. B. Lund, Massachusetts.

The Memorial has also been endorsed by numerous other medical and civic organizations.

Every doctor is requested to take a personal interest in the Gorgas program and to see that his community is adequately represented on the State Governing Committee. Each County Society should appoint officially at least one of its members to serve on the State Committee. This is one foundation that is controlled by the practitioners of curative medicine and as such should be supported by every practicing physician. Let us pull together, "the doctor for the doctor."

Frank Billings
Gilbert Fitz-Patrick
Seale Harris
W. H. G. Logan
Samuel J. Mixter
G. H. de Schweinitz
Rear Admiral E. R. Stitt
George Crile
William D. Haggard
Franklin Martin
William J. Mayo
Stuart McGuire
Ernst A. Sommer
Ray Lyman Wilbur
Surgeon General Hugh S. Cumming
C. Jeff Miller
Major General Merritte W. Ireland
Brigadier General Robert E. Noble
George David Stewart
Hugh Young

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Gorgas Memorial Institute,
Executive Offices: Chicago, Illinois.

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W. P. G. Harding
Judge John Bassett Moore
Adolph S. Ochs
Pres. Beliasario Porras, Panama
Leo S. Rowe
Fred W. Upham

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*How Our Bodies are Made**

THIS surprisingly attractive work on elementary physiology is written, as the title would suggest for "the young idea," when at the age of six or seven it commences to bombard us with questions as to how our bodies are made. The illustrations are readily understood by the age for which the book was written. Thus we see airplanes sailing in through the mouth and moving downward through the air passages into the lungs, where their cargoes are transferred into submarines in the blood and carried through the circulatory channels to remote parts of the body. Or again sailing vessels travelling downward through the esophagus discharging their cargoes at wharves in the intestines where they are met by accommodation trains which travel back through the route of the lymphatics. Even the delicate question as to how people are born is treated in this novel manner, with what the reviewer considers fair success. Bacteria, we find, are black demons with javelins and arches. The defenses of the body are represented by armored soldiers and by the white submarines of the blood. The bodies of these black demons which have been destroyed are placed in the submarines and carried away.

The central nervous system is, of course, represented by a telephone system and is explained in terms of photography, etc. Although there is some risk of overemphasizing the analogies, we feel that in view of the purpose for which the book was written it has been admirably done and will answer satisfactorily the questions proposed by youngsters of early school age.

*How Our Bodies are Made: By R. M. Wilson, M.B., Ch.B. Cloth. Pp. 246. Illustrated. Henry Frowde and Hodder & Stoughton. London.

The American Society of Clinical Pathologists

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Service Bureau

The American Society of Clinical Pathologists maintains a Service Bureau for the benefit of its members which receives requests from hospitals and other institutions desiring the services of a clinical Pathologist. Any member wishing to change his location should communicate with the Secretary.

Eighth Semi-Annual Meeting of the State Pathological Society of Texas

Our colleagues of Texas constitute, in Main Street parlance, a live wire organization very active in promoting the interests of clinical pathology. The State Pathological Society of Texas is a pioneer in the field and has recently held its eighth semi-annual meeting.

The proceedings of their last reunion at Houston, October 8, 1924 covered the following interesting program:

PROGRAM

1. President's Opening Address
   Dr. B. F. Stout, San Antonio, President
2. The Value of Differential Leukocyte Count in Diagnosis. (Lantern Slide Demonstration)
   Dr. M. D. Levy.....Houston
3. The Surgeon and the Pathologist
   Dr. John T. Moore.....Houston
4. Primary Round Cell Sarcoma of the Heart, with Report of Case
   Dr. W. H. Hill and
   Dr. Henry Hartman.....Galveston
5. Is the Pathologist Properly Appreciated?
   Dr. M. F. Bledsoe.....Port Arthur
   President Texas State Medical Association
6. The Last of the Pathologist
   Dr. E. F. Cooke.....Houston
7. Discussion of the Pathological Problems of Today
   Opened by Dr. A. H. Braden..Houston
8. Dinner at San Jacinto Inn